



Inside the stemness engine: Mechanistic links between deregulated transcription factors and stemness in cancer

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ABSTRACT

Cell identity is largely determined by its transcriptional profile. In tumour, deregulation of transcription factor expression and/or activity enables cancer cell to acquire a stem-like state characterised by capacity to self-renew, differentiate and form tumours in vivo. These stem-like cancer cells are highly metastatic and therapy resistant, thus warranting a more complete understanding of the molecular mechanisms downstream of the transcription factors that mediate the establishment of stemness state. Here, we review recent research findings that provide a mechanistic link between the commonly deregulated transcription factors and stemness in cancer. In particular, we describe the role of master transcription factors (SOX, OCT4, NANOG, KLF, BRACHYURY, SALL, HOX, FOX and RUNX), signalling-regulated transcription factors (SMAD, β -catenin, YAP, TAZ, AP-1, NOTCH, STAT, GLI, ETS and NF- κ B) and unclassified transcription factors (c-MYC, HIF, EMT transcription factors and P53) across diverse tumour types, thereby yielding a comprehensive overview identifying shared downstream targets, highlighting unique mechanisms and discussing complexities.

1. Introduction

Cell identity is largely determined by its transcriptional programmes. Transcriptional programmes are driven by master transcription factors that induce the expression of cell type specific genes, silence the genes of other cell types and form autoregulatory circuitries to establish and maintain the cellular phenotype. Accumulating evidence suggests that developmental pathways and genes are reactivated in cancer cells resulting in a stem cell-like state that is responsible for tumour initiation, progression, metastasis and therapy resistance. The stemness state is characterised by the capacity to self-renew, differentiate and form tumours in vivo. It is governed by a small set of aberrantly expressed or activated master transcription factors that are normally involved in the organogenesis and/or maintenance of cell identity. The transcription factors controlling the stem cell-like characteristics can be deregulated by multiple processes: 1) a mutation in the gene body or genomic regulatory element of a master transcription factor, 2) a change in the expression or activity of a protein regulating the expression, stability, localisation or binding of a master transcription factor, 3) oncogenic

signalling, and/or 4) tumour microenvironment (e.g., hypoxia or pathways activated by immune cell-secreted factors)(Table 1).

Importantly, master transcription factors regulate cell type specification and organogenesis which are complex series of events requiring tight coordination between several signalling pathways (e.g., Wnt, TGF- β , Hippo and Notch) and biological processes (e.g., transcription, translation, metabolism, cell cycle and cell migration). To achieve this, master transcription factors cooperate with signal-dependent transcription factors to control the expression of a great number of genes encoding proteins that play a key role in these cellular processes. Large number of target genes and combinatorial action enables transcription factors to cause a profound but specific change in the gene expression profile and transcription factor network. This results in the regulation of many aspects of a cell phenotype and behaviour. Accordingly, mounting data show that in addition to self-renewal capacity and differentiation potential, stem cell-like cancer cells possess many other characteristics that make them the main culprit in cancer. For instance, they are reported to be resistant to radio- and chemotherapy, and show superior migratory and invasive capacity, thus underlying tumour relapse and

Abbreviations: EMT, epithelial-mesenchymal transition; EMT-TFs, EMT transcription factors; ER, endoplasmic reticulum; lncRNA, long non-coding RNA; MET, mesenchymal-epithelial transition; TF, transcription factor; TSS, transcription start site.

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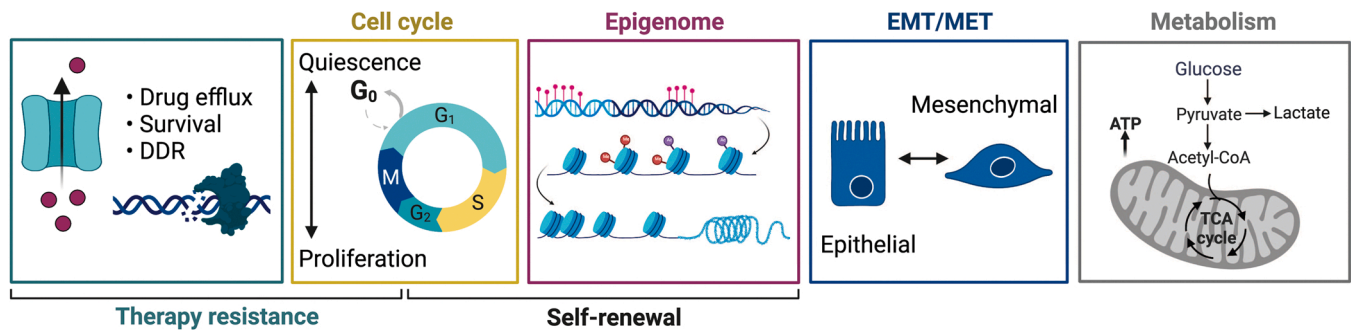


Fig. 1. Definition of stemness in cancer. The stemness state is characterised by the capacity to self-renew, differentiate and form tumours in vivo, and is achieved by tight coordination of cell cycle progression and chromatin dynamics. In addition, stem-like cancer cells are reported to be resistant to radio- and chemotherapy, and show superior migratory and invasive capacity. Therapy resistance is achieved by exporting the therapeutic agents (drug efflux), activation of anti-apoptotic pathways (survival), superior DNA damage response (DDR) and/or acquisition of a quiescent phenotype. Self-renewal is characterised by long-term proliferative potential and undifferentiated chromatin state. Epigenetic features include, among others, DNA methylation, histone modification and higher-order chromatin organisation. Epithelial-mesenchymal plasticity, which facilitates metastatic spread, manifests in reversible conversions between epithelial and mesenchymal states. Reprogramming of cellular metabolism is interlinked with each of the above-mentioned stemness properties. DDR, DNA damage response; EMT, epithelial-mesenchymal transition; MET, mesenchymal-epithelial transition.

metastasis, respectively (Fig. 1).

Altogether, the complex state of stemness is a consequence of oncogenic transformation resulting from transcriptional reprogramming orchestrated by deregulated master transcription factors and facilitated by transcriptional effectors of signalling pathways. In this review, we discuss recent findings on molecular mechanisms linking a selection of deregulated transcription factors to stemness and stemness-associated features in cancer. The stemness-associated features include epithelial-mesenchymal transition (EMT) and therapy resistance. We describe

the role of master transcription factors (SOX, OCT4, NANOG, KLF, BRACHYURY, SALL, HOX, FOX and RUNX), signalling-regulated transcription factors (SMAD, β -catenin, YAP, TAZ, AP-1, NOTCH, STAT, GLI, ETS and NF- κ B) and unclassified transcription factors (c-MYC, HIF, EMT transcription factors and P53).

2. SOX

SOX proteins are developmental master transcription factors that are

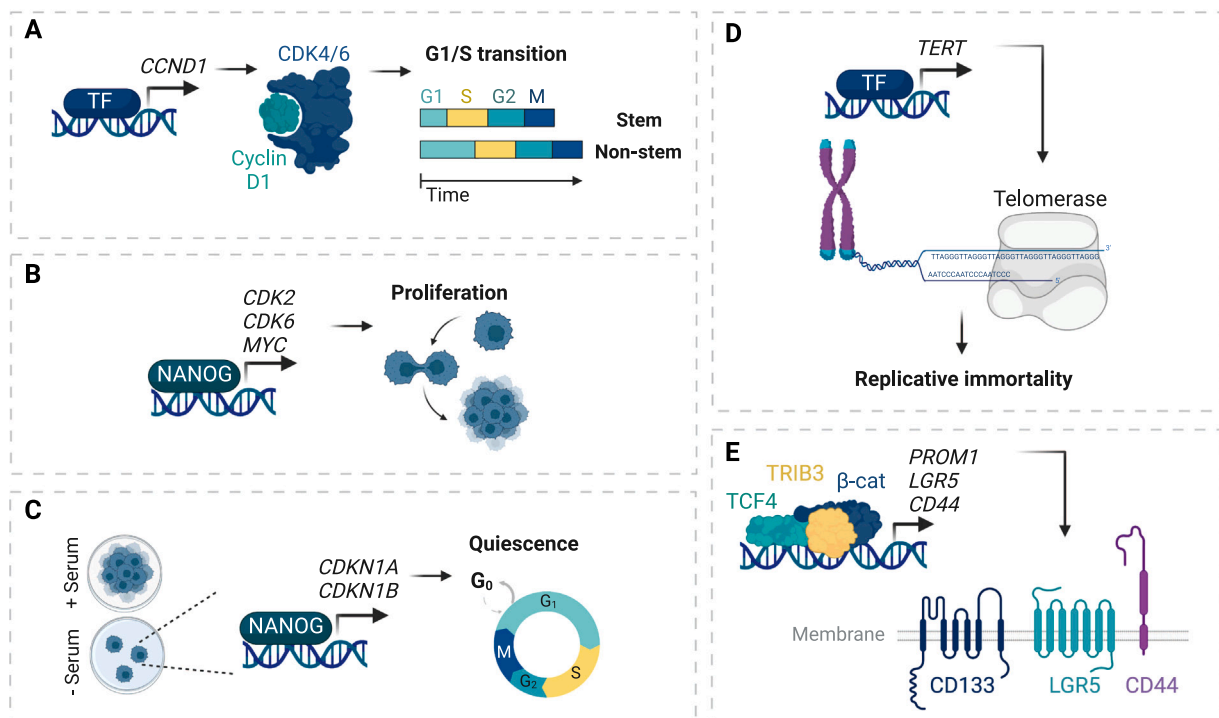


Fig. 2. Regulation of cell cycle and cell surface proteins by stemness transcription factors. A. Transcription factors (e.g., SOX2, SOX4, β -catenin and STAT3) promote transcriptional activation of *CCND1* (encoding cyclin D1) which complexes with CDK4/6 to induce entry into S phase. Upregulation of cyclin D1 in stem-like cancer cells results in shortening of G1 phase. B. NANOG enhances proliferation by transcriptional activation of *CDK2*, *CDK6* and *MYC*. C. Under serum starvation, NANOG induces quiescence by transcriptional activation of cell cycle inhibitors *CDKN1A* and *CDKN1B*. D. Transcription factors (e.g., OCT4, SOX2, MYC, NANOG and β -catenin) promote transcriptional activation of *TERT* (encoding telomerase reverse transcriptase) that endows stem-like cancer cells with replicative immortality. E. Ternary complex of β -catenin, TCF4 and TRIB3 promotes transcriptional activation of cell surface markers *PROM1* (encoding CD133), *LGR5* and *CD44*. β -cat, β -catenin; CDK, cyclin-dependent kinase; CDKN, cyclin-dependent kinase inhibitor; PROM1, prominin 1 (encoding CD133); TCF4, transcription factor 4; TERT, telomerase reverse transcriptase; TF, transcription factor; TRIB3, tribbles pseudokinase 3.

frequently overexpressed in many cancers. All members of SOX family contain an SRY-related HMG-box domain that mediates their binding to DNA. The over 20 factors of SOX family are further classified into 11 subgroups based on the amino acid sequence of HMG domain and overall organisation of protein domains [46,47]. Importantly, SOX proteins alone are not able to regulate gene expression and thus, function by forming a multiprotein complex with partner transcription factors [48,49]. Therefore, their action is highly context-dependent. Furthermore, SOX transcription factors are involved in the regulation of delicate balance between self-renewal and differentiation into diverse cell types which is achieved by tightly regulated expression levels and interplay between the family members (reviewed in [50–53]). Given the role in stemness, cell fate specification, dedifferentiation and reprogramming, the involvement of SOX transcription factors in tumorigenesis is well-recognised and discussed in several reviews [54–56]. Here, we discuss recent research findings that provide a mechanistic link between SOX proteins, stemness and stemness-associated features in cancer.

2.1. Role of SOX transcription factors in self-renewal

Several SOX family transcription factors are important regulators of the stem cell self-renewal. Self-renewal is the process of giving rise to indefinitely more cells of the same cell type. In order to self-renew, the cell has to be able to maintain an undifferentiated state throughout the cell division cycle which highlights the interconnectedness of cell cycle and cell fate choices. The relationship between cell cycle and stemness in cancer appears to be complex as cancer stem-like cells can exist in a quiescent, slow cycling or rapidly proliferating state [57]. Nevertheless, the relationship is unquestionable as master stem cell factors regulate the expression of genes involved in cell cycle and vice versa. SOX2 has been shown to promote the proliferation of cancer cells by inducing S-phase entry [58,59]. SOX2 interacts with β -catenin to upregulate the expression of *CCND1* which encodes cyclin D1, a regulator of G1/S transition (Fig. 2A) [60]. Wnt pathway-dependent induction of proliferation is also utilised by SOX4 as its overexpression resulted in an increase in the protein levels of β -catenin, c-MYC and cyclin D1 in triple-negative breast cancer [61]. Interaction between cyclin-dependent kinase 1 (CDK1) and SOX2 illustrates the reciprocal nature of the relationship. CDK1 is involved in the G2/M transition but it also phosphorylates SOX2. Phosphorylation of SOX2 increases its nuclear translocation, transcriptional activity and in turn the stemness of lung cancer cells [62]. Nevertheless, not all wild-type members of SOX family possess oncogenic potential. For instance, SOX9 and SOX17 induce cell cycle arrest at the G0/G1-phase, and SOX17 reduces the viability of cervical cancer cells [63,64]. SOX17 overexpression leads to downregulation of *CTNNB* (encoding β -catenin), *CCND1* (encoding cyclin D1) and *MYC* (encoding c-MYC) [64]. In accordance with the tumour suppressive function, low levels of SOX17 are associated with advanced stage and poor prognosis in majority of tumour types [65]. Nonetheless, upon acquisition of mutation in the DNA-binding HMG domain, SOX17 becomes a reprogramming factor and is able to induce pluripotency [66].

Directly linked to the cell division is telomere attrition which acts as a major barrier to cancer formation as it allows cells to undergo only a limited number of cell divisions before telomere shortening results in irreversible cell cycle arrest and senescence or apoptosis [67]. However, cancer cells avoid replicative senescence by reactivating the expression of telomerase reverse transcriptase (TERT), a catalytic subunit and rate limiting factor of telomerase complex [68]. Recent evidence shows that stem cell-like cancer cells display higher telomerase activity and longer telomeres than bulk tumour cells. The expression of TERT is induced by the pluripotency factors OCT4 and SOX2, and treatment with small molecule telomerase inhibitor results in decreased expression of stem cell-associated genes and percentage of stem cell-like cells (Fig. 2D). Altogether, the replicative immortality of stem cell-like cancer cells is a

result of an intricate crosstalk between stem cell factors and telomerase [69]. Accordingly, recent analysis of transcriptomes of tumour and non-tumour samples revealed a correlation between active telomerase and cancer stemness program [70]. Furthermore, in addition to its association with replicative immortality, telomerase also affects cancer cell migration, invasion, therapy resistance and stemness suggesting telomerase has roles other than maintenance of telomere length [71].

Undergoing cell division cycle is vital for self-renewal. However, in order to self-renew, the cell also has to maintain the undifferentiated state implying coordination between cell cycle progression and cell fate choice [72,73]. The cell fate decisions are made in early G1-phase of a cell cycle during which many developmental genes contain permissive epigenetic marks (H3K4me3 and 5-hydroxymethylcytosine) [73,74]. On the other hand, in the S- and G2-phase, the stemness state is maintained by actively suppressing differentiation by epigenetic mechanisms [75, 76]. Epigenetic regulators have also been identified as key players mediating the formation and function of cancer stem-like cells [77]. However, the understanding of the interplay between stemness state and cell cycle regulation in cancer is limited. Liu et al. observed direct binding of SOX4 to the promoter of *HDAC1* which promotes *HDAC1* transcription and supports stemness of colorectal cancer cells [78]. HDAC is a histone deacetylase that catalyses deacetylation of histones, leading to a chromatin compaction and transcriptional repression. Therefore, it acts as a part of the co-repressor complex responsible for gene silencing. In addition to modulating the post-translational modification of histones, HDAC is able to deacetylate other cellular proteins (e.g., transcription factors, RNA polymerase and metabolic enzymes) and regulate a wide range of processes implicated in tumorigenesis and therapy resistance (e.g., transcription, metabolism, response to hypoxia, cell cycle, apoptosis, autophagy and DNA damage response) [79]. To conclude, the example of SOX4 demonstrates how a master transcription factor can orchestrate stem cell state by activating the expression of *CCND1* and *HDAC1* to minimise the time in G1-phase and potentially silence differentiation programmes, respectively, as a means to maintain the stemness state [61,78]. Further experimental evidence will help to elucidate the coordination between cell cycle and cell fate decisions in cancer stem-like cells.

2.2. Crosstalk between SOX factors and developmental signalling pathways

Members of the SOX family also modulate the activity and expression of developmental signalling pathways and their components, respectively. SOX2 cooperates with protein kinase C iota (PRKCI) to enhance the expression of Hedgehog acyltransferase (HHAT) that catalyses the rate-limiting step in the synthesis of Hedgehog ligand. Therefore, SOX2 activates autocrine Hedgehog signalling and consequently its downstream effector GLI [80]. In addition, SOX2 binds the promoters of the genes encoding YAP1 and WWTR1 (transcriptional coregulators of Hippo pathway), induces nuclear localisation and transcriptional activity of YAP1, and fails to stimulate stemness in the absence of these cofactors [81,82]. Among the shared target genes of SOX2 and YAP1 in head and neck squamous cell carcinoma is KLF7 which is essential for SOX2-mediated sphere formation capacity [82]. Moreover, the proteins of SOX subfamily E activate Wnt signalling pathway through various mechanisms [83–85]. For instance, SOX9 induces expression of ubiquitin specific peptidase 22 (USP22) which leads to the phosphorylation of β -catenin and activation of Wnt pathway [84,86]. SOX8 and SOX9 bind the promoter region of Frizzled-7 (FZD7), a receptor for Wnt proteins, and induce FZD7-mediated activation of Wnt pathway [85,87]. Altogether, crosstalk with developmental pathways, namely Hedgehog, Hippo and Wnt signalling, appears to play an integral role in the stemness regulation by SOX transcription factors.

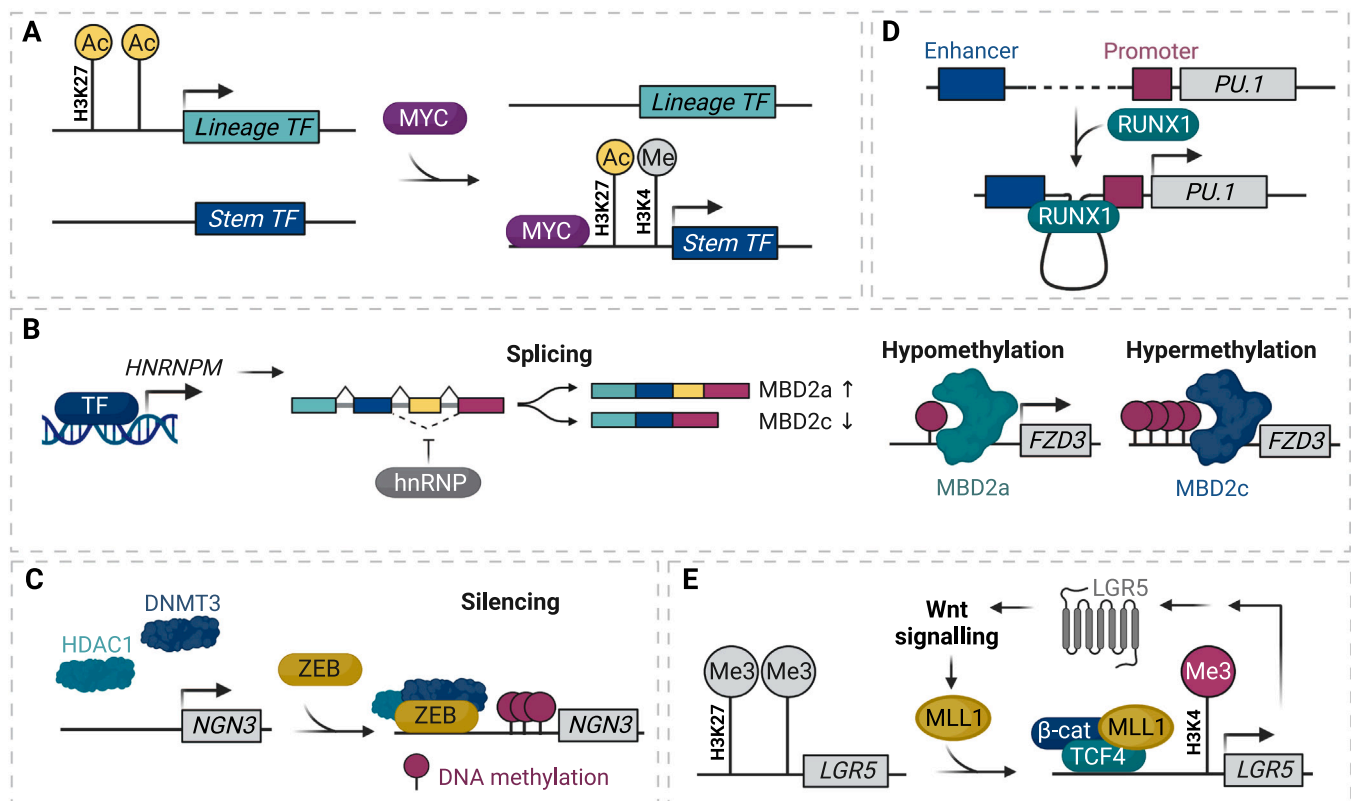


Fig. 3. Regulation of epigenome by stemness transcription factors. A. Upregulation of MYC results in silencing of lineage-specifying transcription factors (accompanied by loss of H3K27ac epigenetic mark) and in activation of stemness-associated transcription factors (accompanied by gain of H3K27ac and H3K4me1 epigenetic marks). B. Transcription factors (e.g., OCT4 and SOX2) promote the expression of *HNRNPM* (encoding a member of hnRNP family) which controls alternative splicing of *MBD2* mRNA. Upregulation of *HNRNPM* leads to increased levels of MBD2a and decreased levels of MBD2c. MBD2a and MBD2c isoforms competitively bind the promoter region of *FZD3* and play opposing roles. Binding of MBD2a promotes DNA demethylation and transcriptional activation of *FZD3*, whereas MBD2c increases the methylation levels and induces gene silencing. C. ZEB1 recruits DNMT3B and HDAC1 to the *NGN3* promoter which leads to promoter hypermethylation and gene silencing. D. RUNX1 activates the transcription of *PU.1* gene by binding to the *PU.1* locus and promoting a formation of chromatin loop between the enhancer and promoter. E. Canonical Wnt/β-catenin pathway upregulates the expression of histone methyltransferase MLL1 which in turn serves as a coregulator for the transcriptional complex of β-catenin and TCF4. Binding of this ternary complex to the promoter of *LGR5* mediates a switch from repressive H3K27me3 to activating H3K4me3 histone modification, thereby inducing transcriptional activation. *LGR5* is a transmembrane receptor that potentiates Wnt signalling. β-cat, β-catenin; DNMT3B, DNA methyltransferase 3B; *FZD3*, frizzled 3; HDAC1, histone deacetylase 1; hnRNP, heterogeneous nuclear ribonucleoprotein; *HNRNPM*, heterogeneous nuclear ribonucleoprotein M; *LGR5*, leucine-rich repeat-containing G-protein coupled receptor 5; Lineage TF, lineage-specifying transcription factor; MBD2, methyl-CpG-binding domain protein 2; MLL1, mixed lineage leukemia 1; *NGN3*, neurogenin 3; Stem TF, stemness-associated transcription factor; TCF4, transcription factor 4; TF, transcription factor.

2.3. SOX2 promotes therapy resistance

In addition to classical stem cell features, cancer stem-like cells have proven to be resistant to conventional chemo- and radiotherapy. Mounting evidence supports a mechanistic link between SOX2 expression and therapy resistance in a number of tumour types [88]. The members of ATP binding cassette (ABC) transporter superfamily endow cells with multidrug resistance as they mediate the efflux of diverse chemotherapeutic compounds from cells [89]. In retinoblastoma and head and neck squamous cell carcinoma (HNSCC), SOX2 overexpression results in upregulation of ABCG2 (Fig. 4A) [81,90]. In glioma, tumour cells acquire resistance to anti-cancer drug BCNU (1,3-bis (2-chloroethyl)- 1-nitrosourea) upon induction of ABCC3 and ABCC6 by SOX2 overexpression [91]. In colorectal cancer, SOX2 promotes chemoresistance by transcriptional activation of ABCC2 expression [92]. Furthermore, inhibition of tumour suppressor networks and pro-apoptotic proteins can promote cell survival and a therapy resistant phenotype. SOX2 enhances survival of glioma cells by inducing the expression of miR-486-5p that targets the tumour suppressor genes *PTEN* and *FOXO1*. Inhibition of endogenous miR-486-5p induces cell death by upregulating pro-apoptotic protein BIM via a PTEN-dependent mechanism [93]. Lastly, SOX2 promotes chemoresistance and stemness

properties by enhancing autophagy via upregulating the expression of Beclin (BECN1), a key component of the pre-autophagic structures [92, 94]. Activation of autophagy using treatment with rapamycin increases chemoresistance and stemness [92].

2.4. SOX transcription factors induce EMT

A large body of research points towards a close association between the EMT programme and stemness features [95]. This notion is further supported by SOX2 and SOX4-induced expression of core EMT transcription factors *SNAIL1*, *SNAIL2*, *TWIST* and *ZEB1* which is accompanied by downregulation of epithelial markers (e.g., E-cadherin), upregulation of mesenchymal markers (e.g., N-cadherin and vimentin) and increased migratory capacity (Fig. 5A) [59,96–98]. Mechanistic studies revealed that SOX2 further enhances EMT by repressing *SNAIL2*-targeting miR-452, whereas SOX4 stimulates expression of methyltransferase *EZH2*, a component of the Polycomb repressor complex 2 (PRC2), which is responsible for silencing the epithelial genes via deposition of H3K27me3 repressive epigenetic mark [97,99]. Additionally, SOX2 also employs a microRNA to alter the DNA methylation and hydroxymethylation patterns that underlie the glioma cell plasticity. In detail, SOX2 stimulates the expression of miR-10b-5p which targets DNA

demethylase TET2 leading to a decrease in 5-hydroxymethylcytosine (5hmC) and an increase in 5-methylcytosine (5mC) levels. In glioblastoma, loss of 5hmC correlates with enhanced stemness and poor prognosis [100]. Finally, it has been suggested that highly metastatic and therapy resistant mesenchymal cancer cells are particularly vulnerable to ferroptosis, a non-apoptotic form of cell death driven by iron-dependent phospholipid peroxidation. This can be explained by cancer cell aberrant metabolism, higher load of reactive oxygen species (ROS) and increased intracellular iron levels (reviewed in [101,102]). Interestingly, expression of SOX2 in lung cancer confers resistance to ferroptosis by upregulating cystine/glutamate antiporter SLC7A11, which is a component of cellular antioxidant system [103].

Taken together, members of SOX transcription factor family govern the stem cell state and the associated features by orchestrating cell division cycle, shaping epigenetic landscape, activating developmental signalling pathways, upregulating drug resistance mechanisms and triggering EMT.

3. MYC

MYC is an oncogenic transcription factor that is activated in majority of human cancers via genetic, epigenetic or post-translational mechanisms [104]. It is also one of the four reprogramming transcription factors that has been used to generate induced pluripotent stem cells from adult fibroblasts [105]. Correspondingly, upregulation of MYC and the resulting epigenetic changes play a central role in the establishment and maintenance of stem cell state in cancer.

3.1. Role of MYC in self-renewal and dedifferentiation

In breast cancer, MYC is a direct transcriptional target of p53 and becomes overexpressed upon loss of p53 as the binding of the latter to the transcription start site (TSS) and 3'-UTR represses the expression of *c-MYC*. The constitutive activation of MYC is necessary and sufficient to maintain stem cell-like phenotype and drive the expansion of the pool of stem cell-like cells. Santoro et al. demonstrated that higher frequency of symmetric divisions underlies the increase in the number of stem cell-like cells upon MYC activation [106]. In addition, Poli et al. showed that MYC drives dedifferentiation of mammary epithelial cells towards a progenitor-like state by inducing an alternative epigenetic program. Upregulation of MYC results in loss of cell identity and acquisition of a stem cell-like state by downregulation of lineage-specifying transcription factors and activation of *de novo* enhancers, respectively (Fig. 3A). The enhancers of genes associated with transcriptional regulatory network of luminal lineage cells show a reduction in the levels of H3K27ac (an epigenetic mark of active enhancers). In contrast, *de novo* enhancers were established by acquisition of H3K4me1 and H3K27ac histone marks as the cells transition to a stem cell-like state upon MYC activation. MYC binding was detected at the *de novo* enhancers and the associated genes show an increased expression. Importantly, the upregulated genes were enriched for stem cell-associated transcription factors and genes involved in activation of Wnt signalling. Accordingly, reactivation of pluripotency-associated transcriptional program upon MYC upregulation endowed cells with long-term self-renewal capacity. Altogether, MYC drives the tumourigenesis, at least in part, by establishing a stem cell-like state via epigenetic reprogramming and reactivation of Wnt pathway. Nevertheless, MYC is not able to bind closed chromatin and thus, relies on pioneer transcription factors that recruit chromatin remodelling complexes to decondense chromatin. Motif enrichment analysis suggests members of FOX and SOX families may function as pioneer factors to enable MYC binding and further epigenetic changes [107]. MYC activation driven dedifferentiation and induction of stem cell markers (e.g., NANOG, OCT4, SOX2 and BMI1) has also been observed in hepatocellular carcinoma. Interestingly, a further increase in *c-MYC* activity decreased the expression of stem cell associated proteins and failed to promote self-renewal. Moreover, the response to

varying levels of *c-MYC* expression is dependent on p53 status. The p53-deficient cells require lower levels of *c-MYC* activation to achieve significant increase in self-renewal than cells harbouring wild type p53 [108]. A similar phenomenon occurs in breast cancer where low expression levels of MYC expand the numbers of mammary stem cells and induce their immortalisation but high levels induce activation of p53 and apoptosis [106]. Therefore, the crosstalk between MYC and p53 in cancer is bidirectional and complex [109].

3.2. MYC controls expression of EMT regulators, TERT and HIF2 α

In addition to direct involvement in the control of self-renewal and stemness, MYC has been implicated in regulating features and factors often associated with cancer stem-like cells. For instance, MYC induces the expression of EMT transcription factors [110,111]. In breast cancer, *c-MYC* interacts with histone acetyltransferase EP300 and histone methyltransferase DOT1L to epigenetically activate EMT regulators. This interaction facilitates deposition of active histone marks (H3ac – acetylated histone H3 and H3K79me – methylated histone H3 lysine 79) at the promoters of *SNAIL*, *ZEB1* and *ZEB2*. The upregulation of transcription factors orchestrating EMT enhances stemness of breast cancer cells [111]. MYC has also been shown to activate the transcription of telomerase reverse transcriptase (TERT) by cooperative binding of TERT promoter and recruitment of chromatin modifying complexes (Fig. 2D) (reviewed in [112]). Furthermore, MYC intersects with hypoxia signalling in T cell leukaemia, where it maintains the self-renewal of cancer stem-like cells by regulating the expression of HIF2- α in cooperation with NANOG and SOX2 [113]. MYC and HIF crosstalk has been reviewed in [114].

3.3. Metabolism and nuanced role of MYC

Despite numerous reports supporting the role of MYC as a factor that promotes cellular reprogramming and self-renewal, accumulating evidence is starting to unveil the complexity and nuance in the regulation of stemness by MYC. In lung cancer, transcription factor ZNF322A promotes self-renewal ability and increases expression of stemness-related genes by transcriptional suppression of *c-MYC*. ZNF322A recruits histone deacetylase to the promoter of *c-MYC* which leads to the downregulation of *c-MYC* and subsequent increase in mitochondrial oxidative phosphorylation and cell motility [115]. Interestingly, cancer stem-like cells of pancreatic cancer also exhibit a distinct metabolic phenotype of mitochondrial oxidative phosphorylation which is acquired through downregulation of *c-MYC*. Mechanistically, suppression of MYC allows increased expression of *PGC1A* which enhances mitochondrial biogenesis, mitochondrial activity, antioxidative capacity, and subsequently low levels of mitochondrial reactive oxygen species (ROS). However, distinct metabolic features are required for stemness but are not sufficient to induce stemness phenotype as suppression of MYC failed to induce the expression of pluripotency genes and enhance the sphere formation capacity of differentiated cancer cells. Furthermore, intermediate levels of MYC endow cells with intermediate metabolic phenotype that is resistant to the treatment with metformin, anti-diabetic drug targeting the mitochondrial complex I [116]. Altogether, MYC expression levels determine the metabolic phenotype rendering it an important player in the molecular machinery mediating metabolic reprogramming/plasticity and thus, supporting cell survival and growth by enabling adaptation to diverse environments. The role of MYC in regulating cancer cell metabolism has been reviewed in [117]. The significance of tight regulation of MYC expression is also exemplified in reprogramming where *c-MYC* induces deacetylation of pluripotency loci by recruiting the histone deacetylase 3 (HDAC3) to respective genomic sequences. This demonstrates the dual function of *c-MYC* as it is beneficial for the early phases of reprogramming but unfavourable later [118].

In conclusion, MYC is a well-recognised oncogenic transcription

factor that acts as a key player in facilitating acquisition of the stemness state by mediating epigenetic reprogramming. Nevertheless, a growing body of research highlights the nuance in the function of MYC as exemplified by the requirement for tight regulation of its expression levels and complex interplay with other transcription factors.

4. KLF

Krüppel-like factors (KLFs) are a family of transcription factors harbouring a triple zinc finger DNA-binding domain at the C-terminus and activation and/or repression domain at N-terminus. The family comprises of 17 proteins which functions are dependent on the cellular context as the same factor can act as a tumour suppressor or oncogene in different types of cancer or at different stages of tumour progression. The expression of KLFs is frequently altered in many cancers, and their roles and regulation have been comprehensively reviewed in [119,120]. However, the molecular links between KLFs and cancer cell stemness are intricate and remain incompletely understood.

4.1. KLF4 as pro-stemness factor

KLF4, a component of the Yamanaka reprogramming cocktail, is the most extensively studied member of the KLF family [121]. It is a multifaceted transcriptional regulator that possesses both transcription activation and repression domains, and is able to recruit both co-activators and co-repressors to specific genomic loci. In addition, considering that its function is dependent on the chromatin environment and proteome of a cell, it is unsurprising that KLF4 has been identified as a factor capable of promoting and suppressing stemness in cancer [122,123]. A number of studies have showed that KLF4 expression enhances stem cell-like properties of breast cancer cells [124–127]. KLF4 upregulates transcription of *ALDH1*, elicits partial mesenchymal-to-epithelial transition (MET) and endows cells with therapy resistant phenotype [124]. *ALDH1*, short for aldehyde dehydrogenase 1, is an intracellular detoxification enzyme whose expression and related retinoic acid signalling have been associated with normal and malignant stem cells [128]. In glioblastoma, positive feedback loop between KLF4 and integrin $\beta 4$ (ITGB4) promotes stem-like cell self-renewal, proliferation and migration. KLF4 binds to the promoter of *ITGB4*, facilitates its transcription and therefore, contributes to increased *ITGB4* expression in glioma. In turn, ITGB4 binding to KLF4 attenuates interaction of KLF4 with its E3 ubiquitin ligase, the von Hippel-Lindau protein, which leads to decreased ubiquitination and accumulation of KLF4. Upregulation of KLF4 results in increased expression of stem cell markers OCT4, NANOG and CD133 [129]. Similarly, KLF4 also promotes expression of *OCT4*, *SOX2*, *NANOG*, *CD44* and *CD133* in colorectal cancer, and depletion of KLF4 impairs sphere formation capacity [130]. Qi et al. demonstrated that KLF4 mediates stemness via activation of p38 MAPK signalling pathway [131]. Additionally, it has been found that KLF4 is dispensable for somatic cell reprogramming and could be replaced by NANOG and LIN28 which together enhance reprogramming by promoting cell epithelialisation [132,133]. This implies that KLF4 may contribute to the reprogramming process partly by inducing MET which is essential for converting mesenchymal fibroblasts into epithelial stem cells. Accordingly, Karagönlü et al. showed that KLF4 upregulation in hepatocellular carcinoma cell line induces reprogramming into stem-like cancer cells that are characterised by expression of epithelial markers (*EPCAM* and *CDH1*) and hepatic stem/progenitor cell markers (*CK19* and *LGR5*). Interestingly, overexpression of KLF4 resulted in higher tumour formation capacity in vivo but reduced migration and invasion capabilities in vitro [134]. This result draws attention to the nature of stemness and stemness-associated features in cancer. Although the stem-like cancer cells often possess superior migratory and invasive capacities, not all stem-like cells are migratory and not all migratory cells are stem-like. Furthermore, accumulating evidence supports the dynamic nature of

the cancer stem-like cell state (also known as plasticity) which manifests in transitions between cellular phenotypes in response to changes in cell environment. Transcription factors are fitting candidates for mediating and coordinating the phenotype changes. To demonstrate, the involvement of KLF4 in acquisition of hybrid epithelial/mesenchymal state and cell epithelialisation implies that dynamic expression of KLF4 may underlie epithelial-mesenchymal plasticity which allows the cells to gain mesenchymal properties at the early stages of metastasis and reverse to epithelial state when colonising the distant site [135].

4.2. KLF4 as anti-stemness factor

Numerous studies confirm the role of KLF4 in inhibiting EMT and activating MET but in contrast to above reports, have found expression of KLF4 to reduce stemness. KLF4 is downregulated in pancreatic cancer, and its expression is positively correlated with E-cadherin and negatively correlated with vimentin and caveolin-1. Several caveolin-1-dependent mechanisms have been implicated in tumour metastasis (reviewed in [136]). Knockdown of KLF4 promotes EMT and facilitates pancreatic cancer cell growth and metastasis in vivo at least in part by derepressing transcription of caveolin-1 gene [137]. In addition to caveolin-1, KLF4 also inhibits transcription of *CD44* and *MSI2* by directly binding the respective promoter regions [138,139]. Depletion of KLF4 increases expression of *CD44* and promotes acquisition of stem-like properties, whereas overexpression induces downregulation of *OCT4*, *NANOG*, *CD133*, β -catenin and vimentin which is accompanied by increased sensitivity to chemotherapy treatment, and inhibition of migration, invasion and metastasis [140]. Similarly, there is a negative correlation between KLF4 and *MSI2*, an RNA-binding protein whose expression promotes pancreatic ductal adenocarcinoma proliferation, migration and invasion, and is associated with poor prognosis [139]. *MSI2* binds mRNA and functions as a translational repressor and inducer of mRNA decay [141]. For instance, it downregulates protein levels of Notch signalling antagonist *NUMB* [141,142]. It also cooperates with *LIN28* to directly induce the mRNA decay of *YAP1* upstream kinases, thereby leading to inhibition of Hippo pathway, activation of *YAP1* and subsequent increase in cancer stem-like cell population [143,144]. Moreover, KLF4 has been identified to promote MET and/or suppress EMT in nasopharyngeal carcinoma, colorectal cancer and non-small cell lung cancer [145–148].

4.3. Diverse roles of other KLF family members

The other members of KLF transcription factor family are less studied but the existing evidence supports their involvement in transcriptional mechanisms that control tumour progression, metastasis and stemness. KLF11 is able to promote EMT by suppressing expression of *SMAD7* (inhibitory SMAD that inactivates TGF- β signalling) which leads to upregulation of *SMAD3* and vimentin. Importantly, transcription of KLF11 is repressed by direct binding of KLF4 to its promoter region [10]. KLF7 is upregulated in pancreatic ductal adenocarcinoma due to transcriptional derepression following inactivation of p53 or as a result of activation of MAP kinase pathway by constitutively active *KRAS*. Inhibition of KLF7 blocks pancreatic ductal adenocarcinoma growth and metastasis by downregulation of IFN-stimulated genes (ISGs, e.g., *IFIT1* and *IFIT3*) and fragmentation of Golgi complex [149]. A recent study highlights the role of *IFIT1* in promoting proliferation, migration, invasion and Wnt pathway activation in pancreatic cancer [150]. In line with this report, *IFIT1* or *IFIT3* knockdown impairs the ability to form colonies in soft agar. Silencing of KLF7 also results in the downregulation of discs large MAGUK scaffold protein 3 (*DLG3*) which has been shown to play an important role in maintaining the integrity of Golgi complex, a membrane-bound organelle in which proteins are further processed and sorted for transport to their eventual destinations (Fig. 5D) [149,151–155]. Fragmentation of Golgi apparatus upon KLF7 knockdown results in reduced secretion of cancer-promoting growth

factors due to reduction in protein glycosylation [149]. In serous ovarian cancer, the protein levels of stemness and EMT markers CD44, SNAIL, ZEB2, vimentin and MMP2 decrease upon silencing of KLF7 [156]. KLF2, which is downregulated in human breast cancer, inhibits tumour growth by controlling the transcriptional activity of the retinoic acid (RA) through modulation of the expression of RA receptors and carrier proteins (Fig. 5E). RA regulates transcription by interacting with two types of nuclear receptors. First, RA receptors (RARs) which inhibit tumorigenesis. Interaction between RA and RAR is facilitated by cellular retinoic acid-binding protein 2 (CRABP2). Second, peroxisome proliferator-activated receptor β/δ (PPAR β/δ) which promote tumorigenesis. Interaction between RA and PPAR β/δ is facilitated by fatty acid-binding protein 5 (FABP5). KLF2 directly induces transcription of RAR γ and CRABP2, and indirectly suppresses FABP5, thereby shifting RA signalling from the pro-tumorigenic to the anti-tumorigenic [157]. This mechanism illustrates how RA, which is produced by ALDH through oxidation of retinal, can promote stemness or differentiation depending on the cellular context [158]. Lastly, earlier studies have revealed that KLF9 functions as a transcriptional repressor and inhibits glioblastoma stemness through downregulation of integrin $\alpha 6$ (ITGA6). KLF9 expression impairs self-renewal capacity and decreases stem cell marker (i.e., BMI1, NESTIN, OLIG2 and SOX2) expression [159].

Altogether, the transcription factors of the KLF family are deregulated in several cancer types, and can either promote or suppress stemness and tumorigenesis depending on the cellular context. Current evidence demonstrates the role of KLFs in regulating several fundamental features of stem-like cancer cells through a number of distinct mechanisms. However, further studies are warranted to uncover the upstream regulators, interaction partners and downstream players of KLFs that modulate their expression and shape their function.

5. OCT4

Octamer-binding transcription factor 4 (OCT4, also known as POU domain, class 5, transcription factor 1 (POU5F1)) is a pioneer transcription factor primarily expressed in pluripotent stem cells and proven to be essential for somatic cell reprogramming into a pluripotent state. It harbours a bipartite DNA-binding POU domain flanked by N-terminal and C-terminal transactivation domains [160]. The two POU subdomains are connected by a linker region that mediates OCT4 interaction with other transcription factors and co-regulators. The interactome defines its target genes and mechanism of action, thereby rendering OCT4 a transcription factor whose function is regulated by the cellular context [161]. Although silenced in somatic cells, OCT4 is frequently reactivated in malignant tissues and promotes stemness in cancer cells [162–166] (reviewed in [167]). Importantly, OCT4 gene has multiple transcript variants, protein isoforms and pseudogenes which may possess divergent functions but cannot be easily distinguished from one another by commonly used molecular biology approaches [168,169]. The detection, expression and function of OCT4 in stem-like tumour cells have been comprehensively reviewed by Wang and Herlyn [169]. Here, we discuss recent evidence elucidating the downstream mechanisms of OCT4 and its pseudogenes in cancer stemness.

5.1. OCT4 cooperates with AP-1, Wnt and TGF- β

OCT4A is the first-identified and most-studied isoform which exhibits exclusive nuclear localisation and has been associated with pluripotency [168,169]. Zhou et al. demonstrated that OCT4A expression enhances self-renewal capacity, promotes cell migration and decreases sensitivity to chemotherapeutic agents in several human cancer cell lines. These effects are at least in part mediated by OCT4-stimulated transcriptional upregulation of FOS [170]. FOS is a proto-oncogene that belongs to the AP-1 transcription factor family and has been shown to promote stemness in head and neck squamous cell carcinoma by upregulating expression of NANOG, MYC, SOX2 and NOTCH1 [171].

Moreover, OCT4 stimulates transcription of another AP-1 family member, JUN, which in turn upregulates the expression of OCT4, forming a positive signalling circuit that is essential for maintenance of the stem-like state in liver cancer [172].

In addition to AP-1 signalling, OCT4 also modulates the activity of canonical Wnt/ β -catenin pathway. In hepatocellular carcinoma, OCT4 overexpression leads to an upregulation of WNT2B (Wnt ligand), FZD4, FZD8 (Wnt receptors) and LEF1 (Wnt pathway transcriptional effector) along with subsequent nuclear accumulation of β -catenin and down-regulation of E-cadherin [173]. Zhu et al. shed light on the potential mechanism that mediates the transcriptional activation of Wnt pathway components by showing that splicing factor heterogeneous nuclear ribonucleoprotein M (HNRNPM) is a direct target of OCT4 and SOX2 (Fig. 3B). HNRNPM interacts with the mRNA of MBD2 and controls its alternative splicing which leads to increased levels of MBD2a and decreased levels of MBD2c. MBD2 is a methyl-CpG-binding domain protein 2 that binds the methylated CpG dinucleotides in the gene promoters. MBD2a and MBD2c isoforms competitively bind the promoter region of FZD3 and play opposing roles. Binding of MBD2a promotes DNA demethylation and transcriptional activation of FZD3, whereas the shorter isoform MBD2c increases the methylation levels and thus, induces gene silencing. Importantly, knockdown of HNRNPM diminished and overexpression of MBD2a enhanced cell migration, invasion, proliferation, survival and sphere formation. Intriguingly, inhibition of HNRNPM also promoted activation of CD8 T cells, implying the role of Wnt/ β -catenin signalling pathway in facilitating the acquisition of stem-like phenotype and creating immunosuppressive microenvironment [174].

Mandal et al. showed that OCT4 cooperates with TGF- β signalling pathway to induce EMT in breast cancer cells. Mechanistically, treatment with TGF- β upregulates the protein levels of OCT4 which thereafter forms a heterodimeric complex with TGF- β pathway transcriptional effector SMAD3 to stimulate transcription of EMT master regulators SNAIL1 and SLUG (Fig. 5A). This increases the expression of matrix metalloproteinases MMP2 and MMP9, decreases the levels of E-cadherin and enhances the chemotactic invasiveness [175]. Furthermore, OCT4 directly activates transcription of CXCL13 whose binding to its receptor CXCR5 further promotes migration and invasion in breast cancer [175,176]. Lastly, OCT4-regulated expression of CD133 endows cells with enhanced tumorigenic capacity in colorectal cancer and non-small cell lung cancer [177,178]. The role of CD133 in cancer stemness is reviewed in [179].

5.2. OCT4 pseudogenes

The pseudogenes of OCT4 have been understudied, however, emerging evidence highlights their tumour promoting functions in several cancer types [180]. The high sequence homology allows the pseudogene to serve as a microRNA sponge that binds the OCT4 targeting miR-145, and thereby upregulates OCT4 expression by preventing its RNA interference-mediated degradation [181,182]. Lettnin et al. showed that silencing of POU5F1B (also known as OCT4-PG1) decreases the expression of OCT4 and ABCB1 which results in low ABCB1 activity and increased sensitivity to chemotherapeutic drugs [183]. In hepatocellular carcinoma, POU5F1B promotes cell proliferation through activation of PI3K/AKT signalling pathway [184].

In conclusion, OCT4 is a well-established component of the core pluripotency circuitry and a number of studies suggest its involvement in the self-reinforcing stemness network of tumour cells. However, the low expression levels and numerous variants have hindered the progress towards understanding the molecular mechanisms that underlie OCT4 pro-tumorigenic and pro-stemness functions. Notwithstanding, existing evidence highlights the crosstalk and cooperation with other stemness pathways as key means whereby OCT4 promotes self-renewal and triggers EMT.

6. NANOG

NANOG is a homeobox transcription factor that is expressed in early human development, silenced in normal adult differentiated cells and reactivated in cancer [185]. NANOG gene has two protein isoforms and 11 pseudo-/retrogenes (*NANOGP1-NANOGP11*) of which only *NANOGP8* possesses a functional open reading frame enabling its translation into a protein [186,187]. NANOG protein harbours a DNA binding domain, transactivation domain, dimerisation domain and nuclear localisation signal [187]. It has a central role in maintaining self-renewal and pluripotency of human embryonic stem cells, and contributes to long-term proliferative potential, metastasis and therapy resistance of stem-like cancer cells as comprehensively reviewed in [186,188,189]. Here, we review recent studies that have helped to further delineate the mechanisms underlying regulation of stemness by NANOG.

6.1. Regulation of cell cycle by NANOG

The importance of coordination between cell cycle and cell fate decision is well-recognised [190]. This coordination is achieved through activation of cell cycle regulators by core stemness transcription factors as exemplified by induction of CDK2, CDK6 and CCND1 (all required for G1/S transition) by NANOG (Fig. 2A–B) [191–193]. In oesophageal squamous cell carcinoma, NANOG promotes proliferation and sphere formation by enhancing expression of IL-6 which in turn leads to phosphorylation and activation STAT3 [192]. In prostate cancer, NANOG stimulates cell cycle entry via upregulation of TGF- β and subsequent phosphorylation of SMAD2 as NANOG knockdown resulted in a G0/G1-phase cell cycle arrest which can be reversed by treatment with TGF- β [194]. In accordance with the above findings and requirement of TERT expression for long-term proliferative capacity, NANOG-positive cells exhibit significantly higher telomerase activity and sphere formation potential than NANOG-negative cells (Fig. 2D) [195]. Interestingly, Zhang et al. demonstrated that NANOG promotes dormancy/quiescence in colorectal cancer cells, which have been subjected to serum starvation (Fig. 2C) [196]. Dormant/quiescent state allows a cell to survive in harsh microenvironment and is functionally defined as a G0/G1 arrest accompanied by lower consumption of glucose and decreased responsiveness to extracellular stimuli. Dormant/quiescent cells are neither dead nor senescent and can re-enter the cell cycle once the conditions improve. Therefore, dormancy/quiescence is a temporary state that enables stem-like cancer cells to escape chemotherapeutics and resume proliferation when the treatment ends [197,198]. NANOG expression increases in serum-free conditions while its knockdown destroys dormant/quiescent state and leads to apoptosis implying essential role of NANOG in establishment of dormant/quiescent state. Mechanistically, colorectal cancer cells rewire metabolism towards enhanced fatty acid oxidation (FAO) in order to adapt to nutrient deprivation. Increased FAO leads to elevated levels of acetyl-CoA which serves as an acetyl donor for lysine acetylation, thus linking metabolism and epigenetics. Accordingly, serum starvation increases the acetylation of H3K27 on NANOG promoter resulting in its transcriptional activation. Thereafter, NANOG promotes dormancy/quiescence by stimulating expression of cell cycle inhibitors *P21* and *P27* through direct binding to their promoter regions. These findings were further supported by very low or no expression of NANOG in regions adjacent to blood vessels which have better nutrient supply [196]. Taken together, regulation of cell proliferation by NANOG is context-dependent as it promotes or inhibits cell cycle progression in diverse cellular backgrounds. Furthermore, the selection pressure imposed by harsh tumour microenvironment triggers the adaptation of cancer cells which results in acquisition of stem-like properties accompanied by evolutionary advantage.

6.2. Promotion of EMT by NANOG

The stem-like cancer cells are often characterised by enhanced migratory and invasive capacities which are acquired by undergoing EMT. NANOG has been shown to modulate the epithelial-mesenchymal plasticity through transcriptional repression of miR-200 that targets EMT-inducing transcription factors *ZEB1* and *SNAIL1* [199] (reviewed in [200]). Moreover, NANOG further enhances the highly invasive mesenchymal phenotype by directly activating the transcription of *SNAIL2*, upregulating the expression of MMP2/9 and downregulating the levels of E-cadherin (Fig. 5A) [193]. In addition to the core EMT transcription factors, higher levels of a chemokine receptor CXCR4 are also observed in cells overexpressing NANOG. Importantly, CXCR4, an orchestrator of cell migration, is a direct target of NANOG in glioblastoma [201,202]. Moreover, NANOGP8, a retrogene that only differs from NANOG in a single amino acid in C-terminal transactivation domain, also promotes expression of EMT transcription factors (PRRX1, TWIST and ZEB) and mesenchymal markers (vimentin and N-cadherin) while downregulating the epithelial genes (E-cadherin) [187,203]. These effects are at least in part achieved by NANOGP8-induced expression of Wnt pathway receptor *LGR5* and subsequent nuclear accumulation of β -catenin. Supporting the notion of a close association between EMT programme and acquisition of stem-like phenotype, overexpression of NANOGP8 results in upregulation of stem cell markers (CD133, CD44, CD54, ABCG2 and MSI1), and enhances gastric cancer cell proliferation, migration, invasion, sphere formation and chemo-resistance [203]. The expression of ABCG2 and CD44 is also regulated by NANOG [204,205]. Furthermore, the reciprocal regulation between core pluripotency factors in human embryonic stem cells is recapitulated by the stem-like cancer cells as NANOG directly stimulates transcription of *OCT4* and *MYC* [191,204].

6.3. Non-canonical function of NANOG

Lastly, contrary to their widely recognised transcription factor function, Mikulenkova et al. observed a considerable amount of NANOG/NANOGP8 in the cytoplasm, where they colocalise with the markers of centrosome [206]. The centrosome is the major microtubule-organising centre (MTOC) which facilitates cell division, vesicle transport and cell signalling, modulates cell polarity and motility, and participates in the maintenance of cell shape [207,208]. The centrosome is duplicated once during a cell cycle yielding functionally different mother and daughter centrosomes, thereby generating an inherent asymmetry between daughter cells [209–211]. Importantly, there is a strong correlation between centrosome age and cell fate, implying the pivotal role of centrosomes in enabling asymmetric stem cell division [211]. Surprisingly, cytoplasmic NANOG/NANOGP8 colocalise with the distal region of the mother centriole and their presence associates with centriole maturation [206]. Notwithstanding, further studies are warranted to elucidate the interplay between NANOG cytoplasmic localisation and mode of cell division.

In conclusion, NANOG and NANOGP8 promote stemness by direct transcriptional control of cell cycle regulators and EMT transcription factors together with activation of developmental signalling pathways and non-canonical cytoplasmic functions.

7. HIF

The hypoxia inducible factors (HIFs) are heterodimeric transcription factors composed of the alpha and beta subunit. There are three isoforms of the alpha subunit (HIF-1 α , HIF-2 α and HIF-3 α) whose expression and stability are controlled by the oxygen concentrations. The beta isoforms HIF-1 β and HIF-2 β (also known as ARNT and ARNT2, respectively) are constitutively present in the nucleus [212,213]. The link between hypoxic microenvironment, HIFs and stemness in cancer is well-established (reviewed in [214–218]). Moreover, enhanced activity of HIF proteins

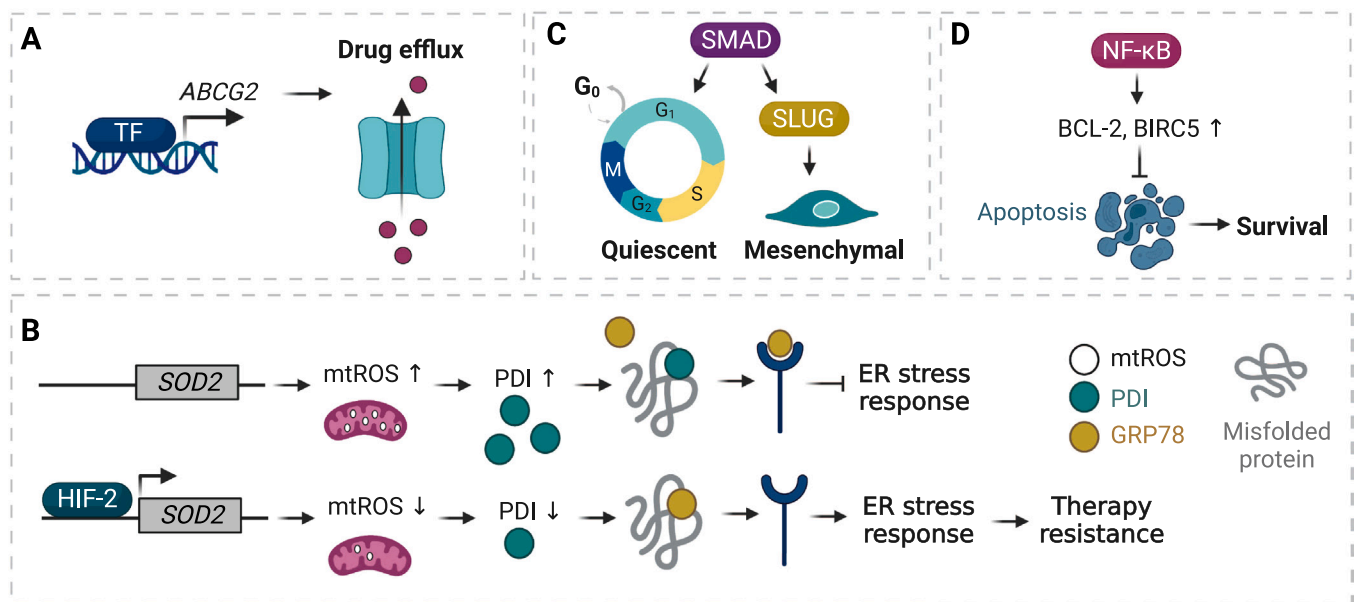


Fig. 4. The role of stemness transcription factors in drug resistance. A. Transcription factors (e.g., SOX2 and SNAI2) promote the expression of ABC transporters (e.g., *ABCG2*) that contribute to chemoresistance through the efflux of anticancer drugs. B. In the absence of *SOD2*, the cell antioxidant capacity is low leading to high levels of *mtROS* and oxidative ER environment which increases the expression and activity of *PDI*. *PDI* is an ER enzyme that competes with *GRP78* for binding to misfolded proteins. An increase in the abundance of *PDI* allows *GRP78* to bind to ER stress sensors and act as an “off switch” for ER stress response pathway. In the presence of *HIF-2*, the *SOD2* gene becomes transcriptionally activated leading to low levels of *mtROS* and downregulation of *PDI*. This results in enhanced binding of *GRP78* to misfolded protein, disassociation of the *GRP78*-ER stress sensor complex and ultimately activation of ER stress response pathway which is involved in the acquisition of chemotherapy resistance. C. In response to treatment with 5-FU, SMAD2 mediates the acquisition of quiescent mesenchymal phenotype. D. NF-κB promotes cell survival by stimulating expression of anti-apoptotic proteins (e.g., BCL-2 and BIRC5). *ABCG2*, ATP-binding cassette transporter G2; BCL-2, B-cell lymphoma 2; BIRC5, baculoviral IAP repeat containing 5 (also known as survivin); ER, endoplasmic reticulum; *GRP78*, glucose-regulated protein 78; *mtROS*, mitochondrial reactive oxygen species; *PDI*, protein disulfide isomerase; *SOD2*, superoxide dismutase 2; TF, transcription factor.

and accompanied expression of hypoxia response genes have also been observed in normoxic tumour cells, wherein HIFs are regulated by oncogenic signalling pathways, a phenomenon called pseudohypoxia [215,219]. The hypoxia response signature includes regulators of pluripotency (e.g., *POU5F1*, *SOX2* and *NANOG*), EMT (e.g., *SNAI1* and *Twist1*), glycolysis metabolism (e.g., *GLUT1*, *GLUT2* and *LDHA*) and drug resistance (e.g., *ABCB2* and *BIRC5*) [215]. All of these processes are of critical importance in endowing cells with tumour- and metastasis-initiating capacity.

7.1. HIF-1α versus HIF-2α

HIF-1α and HIF-2α are the most studied oxygen-regulated transcription factors. They share a high degree of amino acid sequence and structural homology, and bind to the same consensus hypoxia-response element but they possess non-redundant roles, and regulate both common and unique target genes [132,133]. In glioblastoma, *HIF2A* (but not *HIF1A*) is strongly upregulated in stem-like cancer cells under basal and hypoxic conditions compared to matched non-stem cancer cells. Knockdown studies confirm the divergent functions as downregulation of *GLUT1* and *SERPINB9* is specifically detected in *HIF2A* knockdown subclones, whereas only *HIF1A* silencing reduces the expression of *PGK1*. Importantly, loss of HIF-2α in stem-like cells leads to induction of apoptosis, implying the role of HIF-2α downstream targets in supporting cell survival [220]. Nevertheless, both HIF-1α and HIF-2α promote therapy resistance and low proliferation rate by contributing to the upregulation of stem cell factors SOX2 and KLF4 under hypoxic conditions. In turn, SOX2 and KLF4 induce cell cycle arrest in G₁-phase, and stimulate expression of CD133 and CD15, cell surface markers expressed by stem-like cancer cells [221]. Furthermore, SOX2 and HIF-2α-induced OCT4 enhances the self-renewal and migratory capacities of glioma cells by activating transcription of *MENA*. *MENA* is an actin-associated protein that participates in processes involving cytoskeleton remodelling (e.

g., cell migration) [222]. In breast cancer, protein levels of HIF-1α and HIF-2α follow varying dynamics upon exposure to hypoxia. HIF-1α is upregulated rapidly and transiently, whereas HIF-2α levels increase steadily over time [223]. Furthermore, they are both involved in the regulation of stemness but achieve this by different mechanisms. Hypoxia-induced expression of HIF-2α (but not HIF-1α) endows cells with stemness properties through SOD2-*mtROS*-*PDI*/*GRP78*-UPR^{ER} pathway (Fig. 4B). Mechanistically, HIF-2α activates expression of an antioxidant gene superoxide dismutase 2 (*SOD2*), thereby reducing the levels of mitochondrial reactive oxygen species (*mtROS*) [223]. The *mtROS* are generated at the mitochondrial electron transport chain during the process of oxidative phosphorylation and are thereafter transported to other intracellular organelles as signalling molecules [224,225]. Protein disulphide isomerase (*PDI*) is an endoplasmic reticulum (ER) enzyme that competes with glucose-regulated protein 78 (*GRP78*) for binding to misfolded protein and is increased when the ER environment becomes more oxidative. *GRP78* interaction with unfolded protein response (UPR^{ER}) sensors inactivates the UPR^{ER} signalling. In response to less *mtROS* transported to ER, the expression and activity of *PDI* is suppressed which leads to enhanced binding of *GRP78* to misfolded protein, disassociation of the *GRP78*-UPR^{ER} sensor complex and ultimately activation of UPR^{ER} [223]. Activation of UPR^{ER} has been shown to be involved in the acquisition of pluripotency and chemotherapy resistance [226,227]. In accordance with the previous findings, HIF-2α-mediated stimulation of UPR^{ER} signalling confers chemoresistance and stemness properties to breast cancer cells by increased expression of drug efflux transporters (*ABCB1* and *ABCG2*) and pluripotency transcription factors (*OCT4* and *NANOG*). Altogether, hypoxia-induced HIF-2α maintains long-term self-renewal capacity of breast cancer stem-like cells through a novel mechanism involving crosstalk between mitochondria and ER [223]. In contrast, HIF-1α promotes stemness via several pathways, for instance, by inducing transcription of adenosine receptor 2B (*A2BR*), regulating expression of

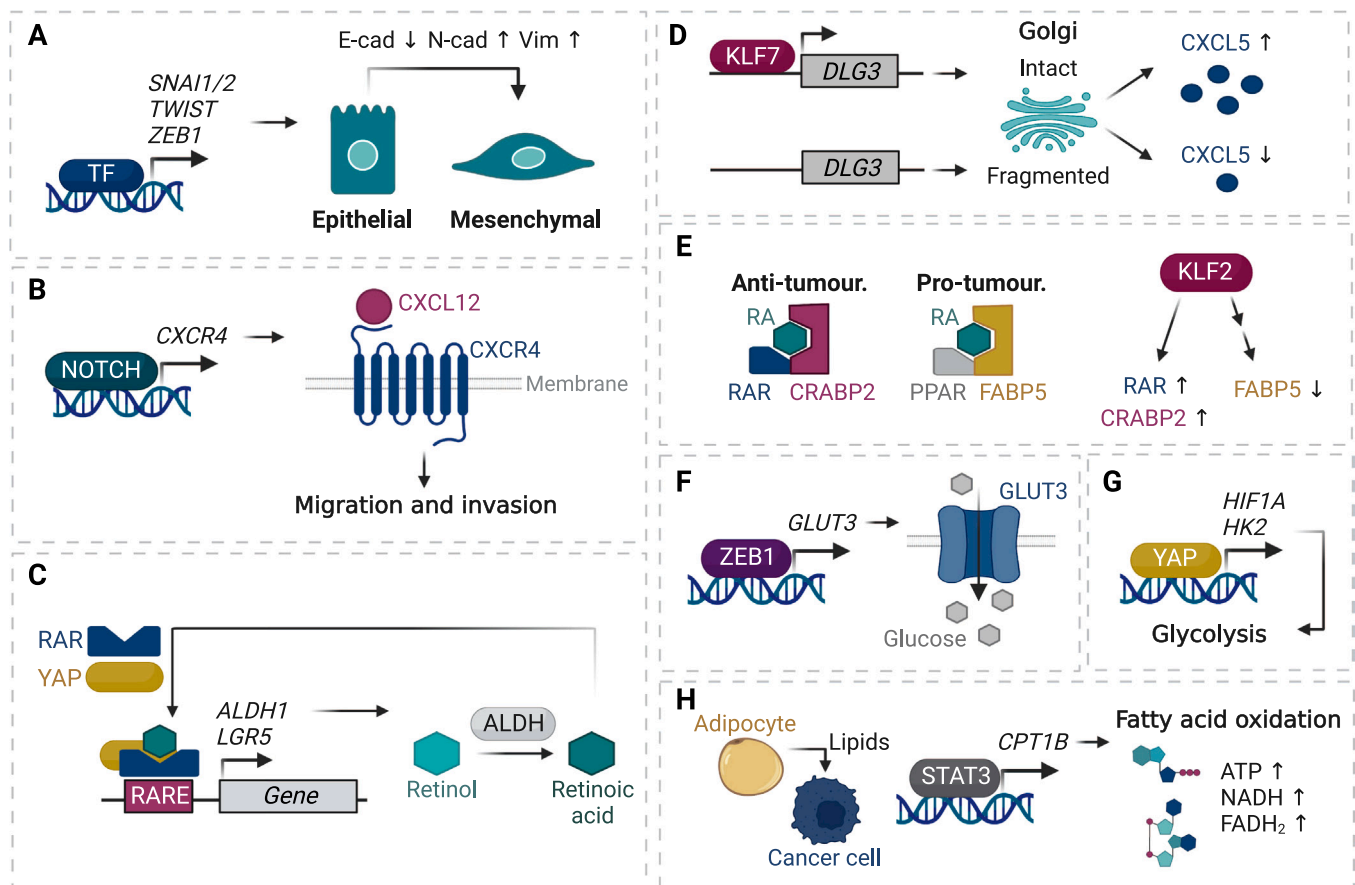


Fig. 5. The role of stemness transcription factors in regulating EMT/MET and cellular metabolism. **A.** Transcription factors (e.g., SOX2, SOX4, OCT4, NANOG, HIF-1 α and HIF-2 α) promote the expression of core EMT regulators (i.e., SNAI1, SNAI2, TWIST and ZEB) which in turn mediate the transition from epithelial to mesenchymal phenotype by downregulating E-cadherin, and upregulating N-cadherin and vimentin. **B.** NOTCH1 enhances cell migration and invasion through upregulation of CXCR4 and activation of CXCL12/CXCR4 signalling. **C.** In colorectal cancer, YAP and RAR α/γ activate the transcription of *ALDH1* and *LGR5* by cooperatively binding to the RAREs of *ALDH1* and *LGR5* promoters. *ALDH1* catalyses the conversion of retinol (vitamin A) to retinoic acid which then serves as a ligand for RAR α/γ . **D.** KLF7 activates the transcription of *DLG3* which plays a key role in maintaining the integrity of the Golgi complex. Fragmentation of Golgi apparatus upon KLF7 knockdown results in reduced secretion of cancer-promoting growth factors (e.g., CXCL5). **E.** In breast cancer, the complex of retinoic acid, CRABP2 and RAR has an anti-tumourigenic role, whereas the complex of retinoic acid, FABP5 and PPAR has a pro-tumourigenic role. KLF2 acts as a tumour suppressor by directly inducing transcription of RAR γ and CRABP2, and indirectly suppressing FABP5. **F.** ZEB1 activates transcription of glucose transporter *GLUT3*. **G.** YAP promotes glycolytic phenotype by upregulating expression of *HIF1A* and *HK2*. **H.** STAT3 activates FAO through transcriptional upregulation of *CPT1B*. FAO is the process whereby fatty acids are broken down to produce energy (ATP), NADH and FADH₂. Exogenous lipid supplies serve as oxidation substrates for FAO. Anti-tumour., anti-tumourigenic; ALDH, aldehyde dehydrogenase; CPT1B, carnitine palmitoyltransferase 1B; CRABP2, cellular retinoic acid-binding protein 2; CXCL, CXC chemokine ligand; CXCR, CXC chemokine receptor; DLG3, discs large MAGUK scaffold protein 3; E-cad, E-cadherin; EMT, epithelial-mesenchymal transition; FABP5, fatty acid-binding protein 5; GLUT, glucose transporter; HK, hexokinase; LGR5, leucine-rich repeat-containing G-protein coupled receptor 5; MET, mesenchymal-epithelial transition; N-cad, N-cadherin; PPAR, peroxisome proliferator-activated receptor; Pro-tumour., pro-tumourigenic; RA, retinoic acid; RAR, retinoic acid receptor; RARE, retinoic acid response element; TF, transcription factor; Vim, vimentin.

dual specificity protein phosphatase 9 (DUSP9) and DUSP16, or enhancing transcription of cyclooxygenase-2 (COX2) [228–230].

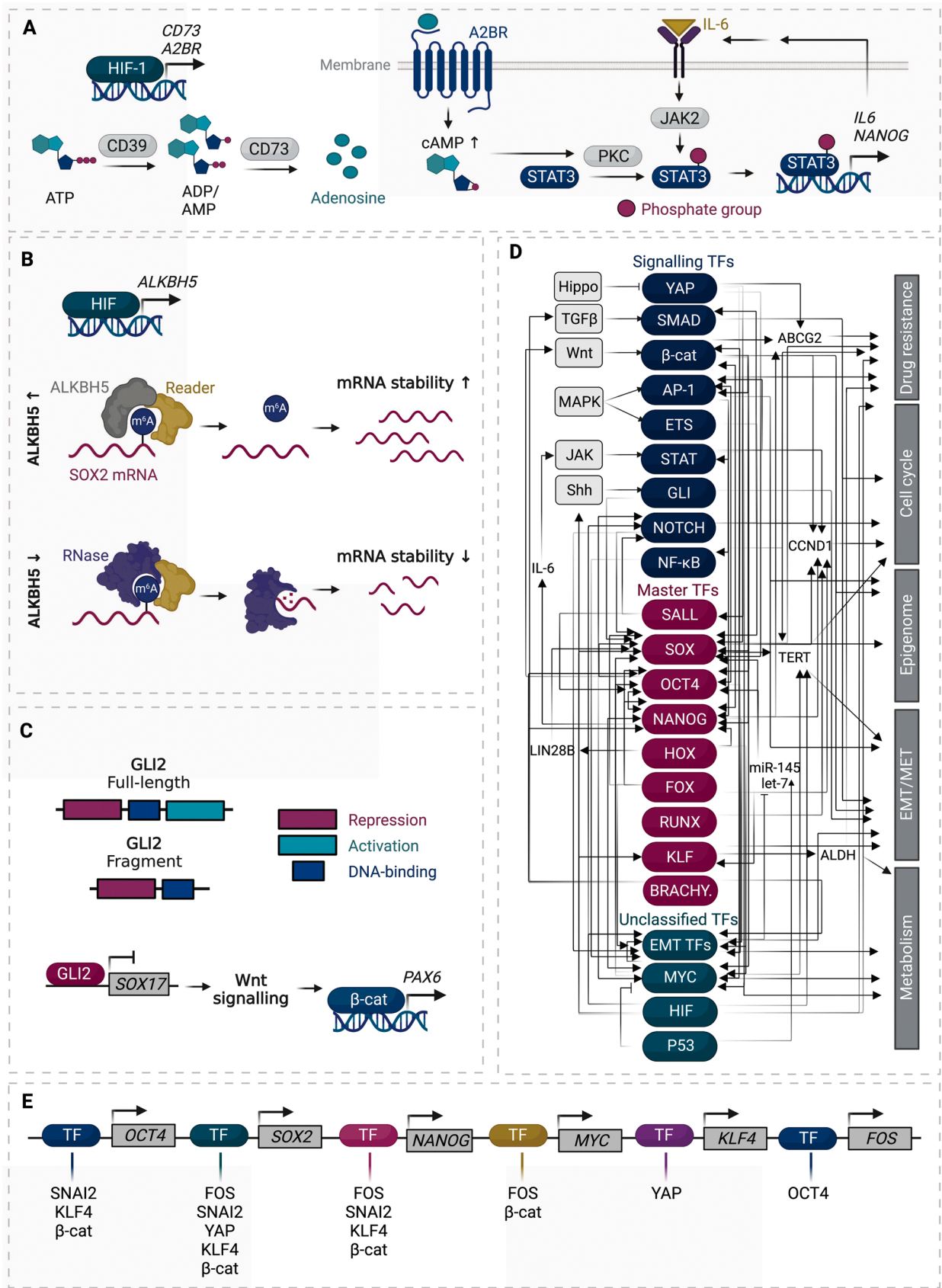
7.2. Crosstalk between HIF-1 α , adenosine signalling, STAT3 and NANOG

Firstly, under hypoxia, extracellular ATP is hydrolysed to ADP and AMP by CD39, and AMP is hydrolysed to adenosine by CD73 [231,232]. Binding of adenosine to its receptor increases production of cyclic AMP (cAMP) which leads to activation of intracellular signal transduction pathways. Samanta et al. have shown that hypoxia upregulates production of adenosine by HIF-dependent activation of *CD73* gene expression in triple-negative breast cancer (Fig. 6A) [233]. Moreover, HIF-1 α binds a regulatory region near the TSS of *A2BR* and directly stimulates its transcription. The activation of *A2BR* by adenosine leads to increased phosphorylation and activation of protein kinase C- δ (PKC δ) which in turn phosphorylates STAT3 at S727. Phosphorylated STAT3 activates transcription of *NANOG* and *IL6*. Once secreted, IL-6

binds to its cognate receptor and activates a tyrosine kinase JAK2 which phosphorylates STAT3 at Y705, a modification that promotes STAT3 nuclear translocation, therefore forming a feedforward loop that amplifies A2BR-mediated signalling. Pharmacological inhibition or short hairpin RNA (shRNA)-mediated knockdown of *A2BR* reduced the number of mammosphere-forming and ALDH-positive cells in vitro, and impaired tumour formation and lung metastasis in vivo, confirming the pivotal role of adenosine-activated pathway in hypoxia-induced enrichment of cancer stem-like cells [228].

7.3. HIF-1 α regulates expression of DUSP9 and DUSP16

Secondly, emerging evidence suggests that disease relapse following chemotherapy treatment is caused by drug-resistant stem-like cancer cells, and chemotherapy itself can promote acquisition of stemness properties by cancer cells. Lu et al. demonstrated that chemotherapy induces enrichment of breast cancer stem-like cells by HIF-1 α -mediated



(caption on next page)

Fig. 6. Crosstalk between stemness transcription factors. **A.** HIF-1 α induces the expression of *CD73* and *A2BR*. *CD73* hydrolyses AMP to adenosine whose binding to its transmembrane receptor *A2BR* increases the production of cAMP and leads to activation of PKC which in turn phosphorylates STAT3. Phosphorylated STAT3 stimulates transcription of *NANOG* and *IL6*. Once secreted, IL-6 binds to its cognate receptor and activates JAK2 which phosphorylates STAT3, thereby completing a positive feedback loop. **B.** HIF-1 α and HIF-2 α promote the expression of *ALKBH5* which increases *SOX2* mRNA stability by catalysing its demethylation. In the absence of *ALKBH5*, m⁶A reader (e.g., *YTHDF2*) recruits ribonucleases that catalyse the degradation of m⁶A-marked *SOX2* mRNA. **C.** The full-length *GLI2* harbours a repression domain, DNA-binding domain and activation domain. The N-terminal fragment of *GLI2* lacks the activation domain and functions as a transcriptional repressor that downregulates expression of *SOX17*. Silencing of *SOX17* leads to upregulation of Wnt signalling pathway and subsequent transcriptional activation of *PAX6* by β -catenin. *PAX6* has been shown to be upregulated upon *GLI2*-mediated transcriptional inhibition of *SOX17*. The involvement of Wnt signalling pathway in linking the expression of *SOX17* and *PAX6* has not been experimentally validated. **D.** Schematic of the molecular network and cellular processes underlying stemness in cancer. The core of the molecular stemness network consists of signalling pathways and transcription factors that modulate the activity of one another. The transcription factors, which are grouped as signalling, master and unclassified regulators, serve as effectors by regulating expression of a number of target genes that encode proteins or non-coding RNAs participating in the stemness network or facilitating acquisition of stemness features. The higher concentration of arrowheads points to key factors commonly regulated in various tumours. The arrow towards a phenotype/process indicates regulation of or effect on a particular feature without specifying the nature of the resulting phenotype. This is a simplified representation that illustrates only a fraction of mechanisms described in the main text. **E.** Stemness transcription factors directly activate transcription of one another. *A2BR*, adenosine receptor 2B; *ABCG2*, ATP binding cassette subfamily G member 2; *ALDH*, aldehyde dehydrogenase; *ALKBH5*, alkB homolog 5, RNA demethylase; β -cat, β -catenin; *BRACHY.*, *BRACHYURY*; cAMP, cyclic AMP; *CCND1*, cyclin D1; *CD*, cluster of differentiation; IL-6, interleukin 6; *JAK2*, Janus kinase 2; *LIN28B*, Lin-28 homolog B; m⁶A, 6-methyladenosine; *PAX6*, paired box 6; *PKC*, protein kinase C- δ ; RNase, ribonuclease; *TERT*, telomerase reverse transcriptase; TF, transcription factor.

regulation of *DUSP9* and *DUSP16* expression. *DUSP9* and *DUSP16* are protein phosphatases that regulate activity of their target proteins through dephosphorylation. In breast cancer cell line, treatment with a chemotherapy drug increases expression of *DUSP9* and decreases expression of *DUSP16* in a HIF-1 α -dependent manner which leads to inhibition of ERK and activation of p38 signalling, respectively. Mechanistically, HIF-1 α stimulates transcription of *DUSP9* which dephosphorylates and inactivates ERK. Inhibition of ERK activity leads to dephosphorylation and nuclear translocation of FOXO3, transcriptional activation of *NANOG* and enhanced stemness of breast cancer cells. In addition, HIF-1 α indirectly represses expression of *DUSP16* by inducing expression of transcriptional repressor *REST*. Lower levels of *DUSP16* lead to activation of p38 which stabilises mRNAs of *NANOG* and *KLF4* through increased inactivating phosphorylation of ZFP36L1, a zinc-finger RNA-binding protein that activates mRNA decay. Ultimately, *DUSP9* knockdown decreases and *DUSP16* knockdown increases the percentage of ALDH-positive cells and the number of mammosphere-forming cells, corroborating opposing roles of the studied phosphatases. Furthermore, the findings illustrate that crosstalk between signalling pathways and transcription factors is bidirectional as transcription factors are able to modulate the activity of signal transduction pathways [229].

7.4. HIF-1 α promotes expression of COX2

Thirdly, HIF-1 α promotes tumour progression by shaping the inflammatory tumour microenvironment through transcriptional activation of cyclooxygenase-2 (COX2, also known as a prostaglandin endoperoxide synthase 2 (PTGS2)), an enzyme responsible for generation of prostanoids (e.g., prostaglandin E2 (PGE₂)) [234]. Ding et al. observed upregulation of stemness-related markers (*SOX2*, *NANOG*, *KLF4*, *SNAIL*, and *BMI1*) and inflammatory genes (*IL6*, *CXCL12*, *IL1B*, *IL10* and *CCL2*) in ovarian cancer cells overexpressing COX2. Furthermore, COX2 upregulation is accompanied by increased proliferative and metastatic capacity [230]. Previous studies have shown that COX2 promotes stemness, proliferation, inflammation, invasion, metastasis and apoptosis resistance mostly through its biologically active lipid metabolite PGE₂ [234].

7.5. HIFs and developmental signalling pathways

In addition to aforementioned novel mechanisms, HIF transcription factors also employ well-established stemness-associated pathways (e.g., developmental Wnt, Notch, TGF- β and Sonic Hedgehog signalling pathways) and processes (e.g., EMT). HIF-2 α overexpression results in activation of Wnt and Notch signalling as protein expression of β -catenin, Survivin (Wnt target gene), Notch^{NICD} (Notch intracellular

domain) and HEY2 (Notch target gene) is significantly increased [235]. Furthermore, HIF-2 α interacts with β -catenin which increases the activity of β -catenin and the protein stability of HIF-2 α , indicating a mutual interaction [236]. Similarly, HIF-1 α also promotes stemness through activation of Wnt and Notch signalling but does so by inducing expression of miR-1275 which directly targets antagonists of Wnt (*DKK3*, *SFRP1*, *GSK3B* and *RUNX3*) and Notch (*NUMB*) pathways. Overexpression of miR-1275 upregulated mRNA levels of *SOX2*, *OCT4*, *ABCG2*, *CD133*, and *ALDH1* [237]. Additionally, HIF-1 α induces Sonic Hedgehog signalling by transcriptional activation of *SHH*, *SMO*, *GLI1* and *GLI2* which leads to increased secretion of SHH and nuclear translocation of GLI1 [238]. *GLI2* transcription is further amplified by coordinated action with SMAD-dependent TGF- β signalling [239]. Activation of developmental pathways and their downstream effectors, namely β -catenin, Notch, GLI and SMAD, regulates stemness through various mechanisms which are discussed below. Notwithstanding, HIF-1 and HIF-2 promote EMT, a process of critical importance in development, wound healing and metastasis that is associated with stemness in cancer, at least in part through mechanisms involving Sonic Hedgehog or β -catenin [236,238]. Overexpression of either HIF-1 α or HIF-2 α increases the expression of EMT transcription factors (*SNAIL* and *SNAIL2*) and mesenchymal markers (vimentin and N-cadherin) but decreases the levels of epithelial markers (E-cadherin), consistent with the acquisition of mesenchymal phenotype with enhanced metastatic capacity (Fig. 5A) [236,238,240–243]. Moreover, hypoxia endows breast cancer cells with increased capability for migration and invasion through HIF-dependent expression of a disintegrin and metalloproteinase domain-containing protein 12 (ADAM12). ADAM12 cleaves off the extracellular domain of the membrane-anchored heparin-binding epidermal growth factor-like growth factor (HB-EGF) which thereafter binds to the epidermal growth factor receptor (EGFR) to activate downstream focal adhesion kinase (FAK)-dependent signalling cascade and promote the metastatic potential of breast cancer cells [244].

7.6. HIFs control cell cycle progression

With regards to the self-renewal, HIF-1 α and HIF-2 α regulate cell cycle progression in a context-dependent manner as they induce cell cycle arrest in some normal cell types and cancer cell lines but enhance cell proliferation in others [245]. For instance, HIF-1 α and HIF-2 α promote cell cycle arrest and low proliferation rate in glioblastoma via induction of *SOX2* and *KLF4* [221]. However, HIF-1 α enhances cell proliferation in pancreatic cancer by transcriptional activation of S-phase kinase associated protein 2 (SKP), a subunit of oncogenic ubiquitin ligase [246]. SKP2 is the substrate recognition component of the SCF E3 ubiquitin ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of, among others, proteins

involved in cell cycle regulation (e.g., cell cycle inhibitors p21 and p27) [247–250]. The protein levels of p21 and p27 decrease in the cells overexpressing SKP2, whereas the stemness-related genes (SOX2, NANOG and LGR5) become upregulated [246]. Furthermore, HIF-2 α promotes proliferative capacity of melanoma cells via miR-363–3p which also inhibits p21 and leads to increased expression of stemness markers CD133, CD271, JARID1B and NANOG [251].

7.7. Cooperation between HIFs and core pluripotency factors

Taken together, the common feature of the above-described mechanisms by which HIFs regulate stemness is induction of expression of transcription factors that have been considered to form the core pluripotency network (i.e., OCT4, SOX2, KLF4 and NANOG) [235,238,240]. To further consolidate the concept, HIF-1 α and HIF-2 α stabilise the mRNA of SOX2 by increasing the protein levels of RNA demethylase ALKBH5 (Fig. 6B) [2]. The 6-methyladenosine (m⁶A) is an mRNA modification that is recognised by m⁶A reader (e.g., YTHDF2) which recruits RNA-degrading enzymes or adaptor proteins to control the stability, splicing and translation of m⁶A-containing mRNA [252,253]. In hypoxia, SOX2 mRNA is upregulated as a result of HIF- and ALKBH5-dependent demethylation [2]. Whereas the majority of the effects of pluripotency transcription factors on cancer cell stemness are independent of HIFs, HIF-1 α is required for NANOG-mediated breast cancer stem-like cell self-renewal as NANOG overexpression failed to induce enrichment of cancer stem-like cells under normoxia. Furthermore, HIF-1 α is not able to induce expression of *TERT* in the absence of NANOG, indicating mutual interaction. Mechanistically, NANOG is recruited by HIF-1 to stimulate transcription of *TERT*. NANOG inhibits degradation of HIF-1 α protein by decreasing its ubiquitination via USP9X-mediated deubiquitination and stabilises HIF-1 α interaction with the coactivator p300, a histone acetyltransferase, which leads to increased levels of H3K9ac and H3K27ac. H3K9ac and H3K27ac are epigenetic histone modifications that have been associated with transcriptional activation. Cooperative activation of *TERT* by HIF-1 α and NANOG is essential for stem cell state as there is positive correlation between stemness index and *TERT* expression/telomere length, and percentage of ALDH-positive cells decreases in *TERT* knockdown clones [254].

In conclusion, HIF-1 and HIF-2 are pleiotropic factors regulating several aspects of the stemness state via distinct molecular mechanisms.

8. SNAI1/2, ZEB1/2 and TWIST

Epithelial-mesenchymal transition (EMT), a shift in a cellular phenotype from an immotile epithelial towards a motile mesenchymal state, plays a central role in the embryonic development and adult tissue homeostasis [255]. EMT programme can be activated by the microenvironmental stimuli which include growth factors, such as epidermal growth factor (EGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF); inflammatory cytokines, such as TGF- β , IL-1 α , IL-1 β , IL-6, TNF- α and IL-8; as well as ligands of Notch, Hedgehog and Wnt signalling pathways. In addition, EMT is triggered by hypoxia, mechanical stress and metabolic changes. These signals subsequently activate the core set of EMT transcription factors (EMT-TFs), which comprises Snail (also known as SNAI1), Slug (also known as SNAI2), Twist-related protein 1 (TWIST1), and zinc-finger E-box-binding homeobox 1 (ZEB1) and ZEB2 (reviewed in [256,257]). Importantly, the abovementioned extracellular triggers together with the additional intracellular regulators are commonly present within various types of tumours resulting in a stimulation of EMT programme in cancer cells. Nevertheless, different cell types express different combinations of EMT-TFs (i.e., tissue-specific EMT-TF code) which may have an influence on the phenotype and behaviour of the cell undergoing EMT [258]. Furthermore, in addition to suppressing the epithelial features and

facilitating acquisition of mesenchymal phenotype, EMT-TFs have been implicated in promoting stemness, therapy resistance and metabolic reprogramming in a context-dependent manner (reviewed in [259–263]). Here, we review recent findings on the interplay between each individual EMT-TF and stemness features in cancer.

8.1. SNAI1/Snail

The main function of EMT-TFs is to downregulate genes involved in cell adhesion (e.g., E-cadherin), and upregulate those responsible for migration and invasion (e.g., vimentin). Accordingly, Snail family transcriptional repressor 1 (SNAI1) is a direct repressor of E-cadherin [264]. Loss of E-cadherin triggers a release of the Wnt pathway effector β -catenin from its complex with E-cadherin leading to a nuclear translocation of β -catenin and transcriptional activation of stemness genes [265]. In non-small cell lung cancer (NSCLC), overexpression of *SNAI1* results in an upregulation of NANOG via SMAD1/AKT/GSK3 β axis accompanied by enhanced sphere formation capacity and chemoresistance [266]. Similarly, SNAI1 promotes stemness and radioresistance in colorectal cancer by inducing expression of NANOG and repressing transcription of miR-145 [267]. The miR-145 is a master regulator of human embryonic stem cell differentiation as it targets pluripotency factors OCT4, SOX2 and KLF4 [268]. In ovarian cancer, SNAI1 downregulates expression of miRNA let-7, which is a well-established tumour suppressor and negative regulator of cancer cell stemness [269,270]. In gastric cancer, stem-like cells exhibit higher expression of SNAI1 which downregulates cellular communication network factor 3 (CCN3) and upregulates neurofilament light chain (NEFL) [271]. The role of CCN3 and NEFL in modulating stemness properties of cancer cells is poorly understood. In breast cancer, TGF- β induces EMT by stimulating upregulation of transcription factors SNAI1 and PRRX1 which have been associated with gain or loss of stem-like features, respectively. Fazilat et al. identified a negative feedback loop, whereby SNAI1 directly represses *PRRX1* and *PRRX1* attenuates the expression of *SNAI1* by activating miR-15 family, therefore demonstrating that the EMT mode is determined by the dominant EMT-TF in a given cellular context. Specifically, elevated levels of SNAI1 manifest in a stemness^{high} EMT phenotype, whereas upregulation of *PRRX1* promotes acquisition of stemness^{low} EMT phenotype [272]. The effects of *PRRX1* are context-dependent as it enhances stemness in glioma [273]. Above observations are in accordance with previous findings underlining continuum of intermediate states between two end points (i.e., epithelial and mesenchymal phenotypes). Importantly, these partial (and transient) states are characterised by varying levels of stemness and orchestrated by transcription factor-driven gene regulatory networks [263].

In contrast to a number of studies reporting pro-stemness functions of SNAI1, Caja et al. revealed an anti-stemness behaviour in glioblastoma as it suppresses self-renewal by transcriptionally repressing the *TGFB1* gene and induces differentiation by activating BMP pathway [274]. In addition, direct downstream targets of SNAI1 in colorectal cancer exhibit significant overlap with the genes induced by master regulators of intestinal stem cells, namely *ASCL2* and *TCF7L2*. As a transcriptional repressor, SNAI1 downregulates the expression of proto-oncogene *MYB* and long non-coding RNA *WNTTLINC1* while silencing the intestinal stem cell signature which together result in attenuated proliferation and impaired clonogenicity [275]. Therefore, EMT and stemness are often linked but can be uncoupled.

8.2. SNAI2/Slug

To date, the Snail family transcriptional repressor 2 (SNAI2) has mostly been shown to exert stemness promoting functions. It is upregulated in CD44-positive stem-like cells of head and neck squamous cell carcinoma, where it increases expression of stem cell markers OCT4, SOX2 and NANOG along with promoting chemoresistance to cisplatin

via stimulating expression of ABC transporters ABCC1–6 and ABCG2 (Fig. 4A) [276]. In pancreatic cancer, SNAI2 enhances self-renewal through regulation of insulin-like growth factor binding protein (IGFBP2) [277]. In leukaemia, loss of SNAI2 impairs self-renewal of stem-like cells by derepression of its direct target *SLC13A3*, a gene encoding a high-affinity sodium-dependent dicarboxylate transporter. *SLC13A3* is involved in cellular energy metabolism and its over-expression results in elevated levels of intracellular reactive oxygen species (ROS) which inhibit cell growth and colony formation in vitro [278]. SNAI2 is also able to promote stemness by a mechanism independent of its transcription factor activity as it interacts with SOX2 in prostate cancer and prevents proteasomal degradation of SOX2 [279].

8.3. TWIST1

Twist family bHLH transcription factor 1 (TWIST1) is a key player in tumour metastasis by inducing EMT. However, expression of TWIST1 during early stages of skin cancer development endows cancer cell with stem-like properties through an EMT-independent mechanism. To illustrate, low levels of TWIST1 are required for initiation and progression of skin papilloma but do not elicit a change in expression of EMT markers, including E-cadherin, N-cadherin and vimentin, indicating its dose-dependent function [280]. Cao et al. showed that TWIST1 promotes metastasis in basal-like breast cancer by directly activating transcription of non-canonical Wnt pathway receptor *ROR1* (reviewed in [281]) [282]. Furthermore, TWIST1 has been reported to bind the proximal E-box element in the *SOX2* promoter and stimulate its transcription [279].

8.4. ZEB

Structurally related transcription factors zinc finger E-box binding homeobox 1 and 2 (ZEB1 and ZEB2) have been identified as regulators of cancer stemness, metastasis and therapy resistance (reviewed in [283–286]). Krebs et al. reported that Zeb1 is a key factor for the formation of precursor lesions, invasion and metastasis in a mouse model of pancreatic ductal adenocarcinoma. Accordingly, depletion of *Zeb1* suppresses stemness and associated phenotypic/metabolic plasticity [287]. Zhou et al. demonstrated that ZEB1 endows breast cancer cells with stem-like properties by inactivating transcription of neurogenin 3 (*NGN3*) (Fig. 3C). Mechanistically, ZEB1 recruits the DNA methyltransferase 3B (DNMT3B) and histone deacetylase 1 (HDAC1) to the *NGN3* promoter which leads to promoter hypermethylation and gene silencing. Restoration of *NGN3* expression attenuates cancer stemness and favours asymmetric cell division [288]. In colorectal cancer, ZEB2 is a master regulator of a mesenchymal and quiescent/slow cycling stem-like state that is resistant to chemotherapy. Ectopic overexpression of ZEB2 upregulates the levels of anti-apoptotic proteins pCRAF and pASK1 along with promoting chemoresistance, stemness, EMT traits and proliferative slowdown [289]. In ovarian cancer, downregulation of ZEB2 reduces cell invasion, colony formation and expression of stemness factors OCT4 and NANOG [290].

8.5. Interplay between EMT, stemness and metabolism

Lastly, stem-like cancer cells have the plasticity to reprogramme their metabolism in order to adapt to adverse microenvironmental conditions (reviewed in [291–293]). On the other hand, the process of EMT is often associated with metabolic changes as cell transitions from a proliferative to a migratory state characterised by distinct energy and biosynthetic requirements. Mounting evidence sheds light on the complex interplay between EMT, stemness and metabolism, wherein EMT-TFs shape the metabolic profile and metabolic enzymes modulate the activity of EMT-TFs (reviewed in [262,294,295]). Furthermore, considering that activation of glycolytic gene signature can accelerate somatic cell reprogramming, and metabolites serve as important

substrates for DNA methylation, histone modification and thereby epigenetic reprogramming, regulation of metabolism by EMT-TFs probably contributes to the enhanced stemness of cells undergoing EMT (reviewed in [296]). For instance, ZEB1 is able to activate transcription of glucose transporter *GLUT3* and inhibition of GLUT3 diminishes tumourigenic potential of brain tumour initiating cells (Fig. 5F) [297,298]. Notwithstanding, further studies are required to elucidate the intricate crosstalk between EMT, metabolism and stemness.

In conclusion, individual EMT-TFs regulate stemness in a dose- and context-dependent manner via mostly unique target genes and downstream mechanisms that are independent of the classical EMT programme. Therefore, it is not the epithelial or mesenchymal state itself but the non-redundant functions of EMT-TFs that facilitate the acquisition of stem-like state.

9. SMAD

The TGF- β /Activin/Nodal developmental signalling pathway has a prominent role in regulating cell growth and differentiation in many tissues, and it has an important function in tumourigenesis. Cells express different combinations of transmembrane receptors of this pathway which upon ligand binding initiate an intracellular phosphorylation cascade. This leads to phosphorylation of the receptor-regulated SMAD transcription factors SMAD2 and SMAD3 which translocate to the nucleus, form a complex with common mediator SMAD4 and cooperate with other sequence-specific transcription factors and co-regulators to modulate transcription of a large number of target genes. The SMAD-containing transcriptional complex exerts its function by binding to specific promoter and enhancer regions and induces or represses the expression of associated genes depending on the composition of the complex. Intriguingly, the target genes and thereby the function of the TGF- β /Activin/Nodal-SMAD2/3 signalling are cell type and context-specific since it can act as a tumour suppressive pathway in untransformed cells and at early stages of cancer development while it acquires a tumour supportive function at later stages of tumourigenesis. This pathway can promote tumour progression through several key processes including cell cycle, EMT, inflammation and chemoresistance, all of which impact the stem-like properties of cancer cells. In particular, the canonical SMAD-dependent pathway is implicated in regulation of cancer cell stemness and SMAD2/3 are aberrantly activated in cancer due to elevated levels of TGF- β in the tumour microenvironment. Next, we will discuss the most recent advances in deciphering the function of SMAD transcription factors in cancer stem-like cells.

9.1. Role of SMAD3 in cancer cell plasticity and partial EMT

EMT is involved in cancer cell metastasis during cell invasion and intravasation, whereas a successful metastatic colonisation requires the inverse process of MET. Importantly, TGF- β /Activin/Nodal signalling coordinates the spatiotemporal regulation of EMT and MET through non-SMAD and SMAD-mediated processes as reviewed in [299]. Earlier studies have shown that the transcription factor Basonuclin-1 (Bnc1) is a direct target of SMAD3 that modulates TGF- β 1-induced epithelial dedifferentiation by affecting the expression of numerous TGF- β 1-responsive genes including distinct EMT-related transcription factors (e.g., c-JUN, FOXA2 and ZEB1). This indicates that Bnc1 is part of a SMAD-regulated transcription factor network governing epithelial plasticity [300]. Junk et al. identified oncostatin M (OSM), a member of interleukin-6 subfamily, as a potent inducer of epithelial-mesenchymal plasticity and stem-like properties [301]. Increasing evidence suggests a link between a transition from epithelial to mesenchymal phenotype and a conversion from non-stem to stem-like state. These phenotype changes are referred to as cellular plasticity whereby tumour cells exploit their developmental potential to acquire a new phenotype in order to adapt to microenvironmental conditions. Cancer cell plasticity promotes

metastasis and facilitates development of drug resistance as reviewed in [302,303]. OSM has been shown to activate STAT3 signalling which in turn promotes SMAD3 nuclear accumulation, DNA binding and transcriptional activity. This endows cells with mesenchymal stem-like phenotype, whereas inhibition of SMAD3 resulted in a reversion to a non-invasive epithelial phenotype [301]. Hence, blocking the STAT3/SMAD3 axis in tumour cells as a therapeutic approach could prevent the plasticity of cancer cells required for metastatic progression and tumour recurrence. In glioma, TGF- β 1 secreted by M2 phenotype tumour-associated macrophages facilitates the stemness and migration of cancer cells by increasing the phosphorylation of SMAD2/3 and the expression of stemness-associated proteins SOX2 and SOX4 [304].

In colorectal cancer, tumour cells tend to invade as cohesive collectives and undergo partial EMT at the invasive front. Partial EMT is characterised by acquisition of mesenchymal transcription pattern while also largely maintaining epithelial gene expression. TGF- β 1 induces partial EMT and promotes collective invasion while abrogating the self-renewal capacity of organoid cells and downregulating intestinal stem cell markers. Mechanistically, induction of the non-progressive partial EMT requires canonical TGF- β signalling mediated by SMADs, whereas the classical EMT master regulators SNAIL1 and ZEB1 are dispensable for partial EMT [305]. The observed decrease in organoid formation capacity is in accordance with the reduction in proliferation which is commonly associated with EMT.

9.2. Role of SMAD proteins in cancer cell quiescence and therapy resistance

Importantly, reversible cancer cell dormancy or quiescence is the main cause of cancer recurrence and failure of therapy since these cells can escape the cytotoxicity of therapeutic drugs. In recent years, TGF β /Activin/Nodal-SMAD2/3 signalling has been shown to regulate cancer stem-like cell dormancy in connection with increased resistance to chemotherapy. Heterogeneous tumours are often chemoresistant due to differences in cell cycle progression among the tumour cells. In squamous cell carcinomas, quiescent tumour-propagating cancer cells resist DNA damage and retain high tumorigenic potential upon chemotherapy, whereas proliferative cells undergo apoptosis. Quiescence is governed by TGF β /SMAD signalling which directly regulates cell cycle gene transcription to control reversible G1 cell cycle arrest. Furthermore, genetic or pharmacological TGF- β inhibition increases the susceptibility of tumour propagating cancer cells to chemotherapy because it prevents entry into a quiescent state [306]. Both SMAD transcription factors and redox imbalance seem to be required for the full induction of a dormancy signature and cell quiescence in prostate cancer [307].

The interplay between TGF- β /SMAD signalling pathway, EMT, proliferation and therapy resistance is further elucidated by Dai et al. work on non-small cell lung cancer (NSCLC). Upon exposure to a DNA damaging agent 5-FU, the surviving NSCLC cells undergo EMT followed by MET. In a subset of cancer cells, treatment with 5-FU triggers a cell cycle arrest in G0/G1-phase coupled with an EMT phenotype, stem-like properties and elevated levels of SMAD2 (Fig. 4C). Upon repair of DNA damage, the dormant cells are reactivated and undergo MET as evidenced by increased ability to proliferate, reacquisition of epithelial phenotype and lower levels of SMAD2. Notwithstanding, further studies are required to better understand the role of SMAD2 in acquisition and dissolution of the dormant, mesenchymal and stem-like state mediating therapy resistance in NSCLC [308]. Nevertheless, high levels of SMAD2 and SMAD3 decrease the sensitivity of breast cancer cells to chemotherapy drugs while blocking SMAD3 phosphorylation could suppress the cancer cell stemness and increase the response to therapeutic agents [309]. Lastly, the common mediator SMAD4 exhibits mostly anti-tumorigenic features and is frequently deleted or mutated in pancreatic cancers cells. SMAD4 also decreases the population of ALDH1A1 positive pancreatic cancer initiating cells through transcriptional repression of *ALDH1A1* [310].

In conclusion, the SMAD transcription factors play an integral role in stem-like cancer cells where they facilitate cellular plasticity, mediate EMT, regulate cell cycle and promote therapy resistance.

10. β -catenin

The β -catenin (encoded by *CTNNB1*) is a multifunctional protein whose structure enables interaction with a great number of proteins (e.g., E-cadherin, MED12, EP300 and members of TCF/LEF family) and thereby facilitates the structural and signalling roles of β -catenin (reviewed in [311]). In this review, we focus on the role of β -catenin as a transcriptional effector of canonical Wnt signalling pathway. Upon binding of a Wnt ligand to its transmembrane receptor Frizzled and co-receptor LRP5/6, β -catenin accumulates in the cytoplasm, translocates to the nucleus and functions as a regulator of transcription. To emphasise, β -catenin does not possess a DNA-binding domain and forms a complex with TCF/LEF transcription factors in order to regulate expression of specific target genes (reviewed in [312]). Therefore, β -catenin is not a transcription factor by definition but is included in this review given its frequent upregulation in cancer and role in modulating stemness through transcriptional regulation [313,314].

10.1. β -catenin activates expression of stemness and EMT markers

β -catenin contributes to the cancer cell stemness by directly or indirectly upregulating the expression of core pluripotency transcription factors, stem cell markers and genes associated with EMT. In breast cancer, β -catenin induces expression of OCT4, SOX2, c-MYC, NANOG, N-cadherin, vimentin, SNAIL2, ABCB1, ABCG2, CCND1 and c-JUN, thereby promoting EMT, proliferation and chemoresistance along with stemness [315,316]. In turn, c-JUN directly activates the transcription of miR-5188 which binds the mRNA of FOXO1 and mediates down-regulation of FOXO1 protein levels. This serves as a positive feedback loop as FOXO1 would otherwise interact with β -catenin to trigger its ubiquitin-dependent degradation and prevent its nuclear accumulation [315]. Similarly, β -catenin upregulates expression of OCT4, SOX2 and NANOG also in liver cancer [317,318]. Additionally, Chen et al. showed that β -catenin-mediated stimulation of stem cell genes may be further enhanced by crosstalk with TERT. TERT, a direct transcriptional target of β -catenin, increases the expression of OCT4, NANOG, SOX2 and CD133, and promotes nuclear accumulation of β -catenin. Importantly, β -catenin and TERT interact to form a protein complex, and over-expression of TERT is able to restore the radioresistant phenotype abolished by inhibition of Wnt/ β -catenin signalling. These findings exemplify the previously underappreciated non-canonical roles of TERT that also involve interplay with other regulators of stemness [319]. In colorectal cancer, β -catenin is required for enhanced expression of MMP9, CD133, CD44, c-MYC, vimentin and periostin [320,321]. Periostin is a matricellular protein, a non-structural element of extracellular matrix (ECM), that facilitates the maintenance and metastasis of cancer stem-like cells by being a component of the stem cell and metastatic niches, respectively [322,323]. It is known to be involved in the acquisition of many hallmarks of cancer as reviewed in [324]. Importantly, periostin promotes sphere formation, and expression of CD133 and CD44 in colorectal cancer [321]. Ma et al. showed that periostin activates FAK-Src kinases through integrin-mediated signalling which results in the activation of Hippo pathway effectors YAP/TAZ and subsequent upregulation of IL-6 expression [325]. A number of independent reports have described the stemness-enhancing effects of IL-6 on cancer cells via activation of the STAT3 pathway [326–329]. Furthermore, activation of YAP/TAZ also promotes stemness through various downstream effectors (reviewed in [330]).

10.2. β -catenin cooperates with epigenetic modifiers

β -catenin induces expression of and cooperates with epigenetic

regulator MLL1 to promote the active state of stem cell genes (Fig. 3E). MLL1 is a histone methyltransferase that is responsible for deposition of H3K4me3 epigenetic mark, a chromatin modification that is enriched at the TSS of active genes. MLL1 expression is upregulated by canonical Wnt/ β -catenin pathway, and loss of MLL1 results in decreased sphere formation in vitro and tumour formation in vivo. Mechanistically, upon depletion of MLL1, histone modification at the stem cell promoters switches from activating H3K4me3 to repressive H3K27me3, indicating that MLL1 sustains stem cell gene expression by antagonising polycomb repressive complex 2 (PRC2)-mediated gene silencing. Interestingly, in the absence of MLL1, the complex of β -catenin and TCF4 binds the promoter of stemness regulator *LGR5* but fails to induce its transcription. Therefore, β -catenin requires MLL1 as a co-factor in order to enhance cancer cell stemness [331].

10.3. β -catenin increases cellular plasticity and therapy resistance

In addition to maintenance of the stemness state, β -catenin also participates in phenotypic plasticity by facilitating the acquisition of stem cell-like properties in lymphoma through Wnt/ β -catenin/c-MYC/SOX2 axis. In response to oxidative stress, the activity of Wnt/ β -catenin pathway is upregulated which results in overexpression of c-MYC and subsequent increase in transcriptional activity of SOX2. In turn, SOX2 stimulates transcription of *WNT2B* and *CTNNB1*, therefore completing a positive feedback loop that reinforces the therapy resistant phenotype also characterised by enhanced self-renewal capacity [332,333]. Notwithstanding, the interplay between c-MYC and SOX2 appears to be dependent on tumour type as c-MYC directly activates the transcription of *SOX2* in colorectal cancer but enhances the binding of SOX2 to genomic regulatory regions without changing its protein levels in lymphoma [320,332].

10.4. β -catenin employs adaptor proteins

Lastly, the transcriptional role of β -catenin relies on its interaction with TCF/LEF transcription factors at specific genomic loci. Recent studies have elucidated the dynamics of the complex formation and indicate the requirement of additional proteins that act as an adaptor to facilitate recruitment of β -catenin to genomic regulatory element or TCF/LEF to β -catenin. In colon cancer, DBC1 enhances β -catenin-dependent and LEF1-mediated transcriptional activation of *MACC1* by stabilising the interaction between LEF1 and *MACC1* enhancer, and facilitating subsequent recruitment of β -catenin. Thereafter, β -catenin and DBC1 participate in long-distance chromatin looping that brings together the promoter and distal enhancer of the *MACC1* locus. Importantly, *MACC1*, short for metastasis-associated in colon cancer 1 (also known as MET transcriptional regulator), plays a critical role in self-renewal capacity of colon cancer cells as its knockdown decreased the number and size of the tumour spheres [334]. *MACC1* is frequently overexpressed in colorectal cancer and promotes proliferation, EMT, stemness and chemoresistance by activating transcription of protumorigenic genes including *MET*, *OCT4*, *NANOG* and *ABCB1* [335–338]. In addition, β -catenin employs tribbles pseudo-kinase 3 (TRIB3) as an adaptor that facilitates the β -catenin-TCF4 complex formation. Depletion of TRIB3 suppresses the recruitment of β -catenin-TCF4 complex to the promoter region of genes regulated by Wnt, and disruption of the interaction between β -catenin and TRIB3 decreases the expression of stemness genes (*CD133*, *CD44*, *LGR5* and *ALDH2*), impairs sphere formation capacity and inhibits cell viability and migration (Fig. 2E). To ensure the presence of pivotal interaction partner, β -catenin-TCF4 complex stimulates transcription of *TRIB3* gene and β -catenin inhibits ubiquitin-proteasome system-dependent degradation of TRIB3 protein [339].

In conclusion, β -catenin is a multifaceted player that exerts protumorigenic effects on cancer cell by stimulating expression of both known and novel stemness regulators through transcriptional and

epigenetic mechanisms. The function of β -catenin is dependent on the cooperation with interacting proteins and downstream effector pathways, and is reinforced by multiple positive feedback loops. As a result, expression of β -catenin endows cells with enhanced tumourigenic and metastatic capacities.

11. YAP and TAZ

The paralogs Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ, also known as WWTR1) are major downstream effectors of the Hippo pathway which plays a key role in the development and tissue regeneration. YAP and TAZ are structurally similar but not identical transcriptional coactivators that lack DNA-binding domain and hence partner with DNA-binding transcription factors of TEAD or AP-1 family. Similarly to β -catenin, YAP and TAZ are included in this review due to their frequent deregulation in cancer and role in modulating stemness through transcriptional regulation [340]. The expression and activity of YAP and TAZ in human cancers is elevated by various mechanisms such as YAP gene amplification, inactivation of Hippo signalling, mutation in upstream G protein-coupled receptor (GPCR), constitutive activation of KRAS, gene fusion or hyperactivation of other signalling pathways (e.g., Wnt, TGF β and Notch) [341–343]. Subsequently, aberrantly activated YAP/TAZ regulate expression of genes involved in cell fate specification and predominantly facilitate acquisition of stemness properties [343,344].

11.1. YAP and TAZ upregulate stemness markers

The proteins of YAP and TAZ exhibit structural differences and their functions do not completely overlap, however, they both have been reported to induce expression of stemness markers in various tumour types [345]. In oesophageal squamous cell carcinoma, the genes encoding YAP and TAZ are frequently amplified, whereas the Hippo pathway kinases, negative regulators of YAP and TAZ, are commonly deleted or mutated. The elevated expression of YAP increases the protein levels of SOX2, SOX9 and NANOG, and the activity of ALDH1 [346,347]. Wang et al. demonstrated that YAP and platelet-derived growth factor (PDGF) signalling form a positive feedback loop that enhances tumourigenicity and chemoresistance of bladder cancer cells. Accordingly, YAP partners with TEAD1 to trigger transcription of platelet derived growth factor subunit B (*PDGFB*) which in turn binds to and activates PDGF receptor (PDGFR). Activated PDGFR interacts with YAP and inhibits its phosphorylation by upstream Hippo kinases, thereby stabilising YAP and promoting its entry into the nucleus [348]. Importantly, YAP plays a central role in bladder cancer as its downregulation inhibits expression of *ALDH1A1*, *CD44*, *CD133*, *SOX2* and *KLF4*, and impairs self-renewal of stem-like cells [348,349]. In lung cancer, TAZ induces transcription of *ALDH1A1* by directly binding its promoter region [350]. In head and neck squamous cell carcinoma, the complex of TAZ and TEAD4 interacts with SOX2 promoter and facilitates its transcription. This leads to elevated protein levels of SOX2 and subsequent upregulation of *CD44*, *CD133* and *ALDH1A1* [351]. In gastric cancer, silencing of YAP reduces the expression of *OCT4*, *NANOG*, *SOX2*, *SALL4* and *LGR5* [352]. Further, YAP promotes colony formation and invasion by transcriptionally and post-transcriptionally upregulating expression of *MYC*. Mechanistically, YAP binds to the enhancer regions of *MYC* to control its transcription and modulates the activity of miRNA processing machinery to control translation of *MYC* mRNA [353]. Lastly, YAP has also been reported to upregulate expression of *ABCG2* [354].

11.2. YAP and TAZ facilitate cellular plasticity

In addition to the role of YAP and TAZ in the maintenance of stem-like state, mounting evidence highlights their involvement in the cellular dedifferentiation and plasticity. Dedifferentiation is a process whereby differentiated cells become less specialised and reacquire stem/

progenitor-like properties. Castellan et al. showed that YAP/TAZ are master regulators of stemness and cell state transitions as their inhibition locks differentiated glioblastoma cells into non-tumourigenic state while YAP/TAZ-positive cells are able to revert to stem-like state. To illustrate, YAP/TAZ-depleted cells retain expression of GFAP, a marker of astrocyte-like cells, in the stemness-promoting medium and fail to form gliomaspheres which confirms that YAP/TAZ are essential for plasticity and self-renewal. In addition, inactivation of YAP/TAZ caused a loss of FOS, FOXO1, FOXO3, SALL1, BCL6 and ERF expression [355]. In pancreatic cancer, differentiated cancer cells dedifferentiate into stem-like cells following radiotherapy. Zhang et al. demonstrated that high mobility group box protein 1 (HMGB1) is released from tumour cells upon exposure to X-ray irradiation leading to activation of YAP which interacts with HIF-1 α to directly stimulate transcription of *OCT4*, *SOX2*, *NANOG* and *MYC*. Downregulation of YAP reduced the expression of stemness markers CD133, *NANOG* and *SOX2*, and diminished the sphere-forming ability [356]. In accordance with these findings, Yan et al. reported that activation of YAP in pancreatic cancer leads to upregulation of *NANOG*, *OCT4* and *SOX2* along with enhancing the sphere formation ability. Moreover, YAP is involved in metabolic reprogramming as it promotes glycolytic phenotype evidenced by increase in expression of HIF-1 α and hexokinase 2 (HK2), and elevated levels of lactate (Fig. 5G) [357]. The ability to rewire and adapt cellular metabolism plays a key role in stemness as it allows cancer cells to survive and thrive in diverse microenvironments (reviewed in [358, 359]).

11.3. YAP enhances cell migration and invasion

Therefore, metabolic plasticity is essential for successful completion of metastatic cascade and outgrowth in distant organs. In addition, cancer cells must be able to intravasate and extravasate in order to spread to a secondary site. Liu et al. showed that YAP cooperates with TEADs and transcription factor PRDM4 to induce expression of leukocyte-specific integrin *ITGB2* in cancer cells. *ITGB2* promotes adhesion to endothelium and facilitates tumour cell transendothelial invasion [360]. Chang et al. reported that YAP induces activation of NF- κ B which contributes to the upregulation of *SNAI2*, enhances EMT and promotes cell metastasis. The increased migratory and invasive capacity is accompanied by elevated expression of stemness markers *SOX2*, *OCT4*, *NANOG*, *KLF4*, *ALDH1A1* and *CD44* [361]. In lung cancer, silencing of YAP inhibits cell migration, invasion and sphere formation along with reducing expression of mesenchymal markers N-cadherin and vimentin. Mechanistically, downregulation of YAP1 diminished the protein levels of TGF- β and TWIST, and reduced the phosphorylation of SMAD2 while inhibition of TGF- β pathway decreased the expression of YAP1, indicating a crosstalk between YAP1 and TGF- β signalling [362].

11.4. The role of YAP/TAZ in colorectal and breast cancers

The role of YAP in colorectal cancer remains controversial. A number of studies have found YAP to promote stemness as its expression enhances sphere formation and migration, whereas its knockdown results in downregulation of stemness (*SOX2*, *ALDH1*, *LGR5*, *OCT4*, *CD44* and *CD133*) and EMT (*SNAI2* and vimentin) markers [363,364]. Bauzone et al. described a mechanism whereby YAP interacts with retinoic acid receptors RAR γ and RXR α to activate transcription of *ALDH1A3* and *LGR5* (Fig. 5C). Importantly, YAP, RAR α , RAR γ , and RXR α co-occupy retinoic acid response elements (RAREs) of *ALDH1A3* and *LGR5* promoters, and YAP activation increases the mRNA and protein levels of *ALDH1A3*, *LGR5* and *OCT4*. In contrast, silencing of YAP, inhibition of RAR/RXR or depletion of vitamin A diminished self-renewal and downregulated stem-like properties (including chemoresistance) confirming the central role of YAP in the self-amplifying retinoic acid signalling. To clarify, aldehyde dehydrogenase (ALDH) catalyses the conversion of retinol (vitamin A) to retinoic acid that then serves as a

ligand for retinoic acid receptors [363]. Notwithstanding, Cheung et al. showed that YAP activation reprogrammes stem-like colorectal cancer cells into Klf6⁺Lgr5⁺ non-proliferative state characterised by low Wnt activity and reduced stemness. Accordingly, activation of YAP leads to tumour regression and YAP-positive cells are less able to seed and establish metastasis in the liver. The conflicting results on the effect of YAP expression on colorectal cancer tumour propagating potential may be explained by the different study systems (in vitro or in vivo) and models (developmental YAP deletions or acute, adult and colon-specific manipulation of YAP) used [365].

In breast cancer, the complex of YAP and RUNX1 binds to the promoter of histone deacetylase 2 (*HDAC2*) and activates its transcription to induce chemoresistance and stemness. Silencing of YAP decreases the mRNA and protein levels of *HDAC2*, and downregulation of *HDAC2* reduces the subpopulation of stem-like CD44^{high}/CD24^{low} cells, impairs mammosphere formation and reverses chemoresistance. In addition, YAP cooperates with TEAD1 to promote transcription of *SRGN* which encodes a small glycoprotein serglycin that is overexpressed in multidrug-resistant breast cancer cells. Moreover, elevated serum SRGN levels correlate with poor response to chemotherapy. The secreted SRGN activates ITGA5/FAK/CREB signalling to upregulate transcription of YAP, therefore, forming a positive feedback loop [366]. Yang et al. showed that TAZ is essential for the maintenance of telomeres in triple-negative breast cancer (TNBC) cells where it maintains telomere length by activating transcription of *RAD51C*, a component of homologous recombination machinery that mediates telomere replication. Knockdown of TAZ leads to a significant decrease in the telomere length and causes a senescence phenotype [367]. Previous studies have revealed that the mRNA levels of TAZ are higher in TNBC than in other breast cancer subtypes and TAZ expression endows breast cancer cells with stem-like features [368–370]. This suggests that TAZ promotes stemness in TNBC at least in part by regulating telomere integrity.

In conclusion, the Hippo pathway transcriptional effectors YAP and TAZ integrate mechanical, metabolic and signalling inputs, and exhibit predominantly pro-stemness functions in various tumour types as exemplified by their role in upregulating stem cell markers, facilitating cell state transitions, promoting cell migration and invasion, enhancing therapy resistance and supporting telomere integrity.

12. AP-1

Activator protein 1 (AP-1) is a dimeric transcription factor complex that is composed of the members of JUN, FOS, ATF and MAF family. They belong to a group of basic leucine zipper (bZIP) proteins as they harbour a basic domain for interaction with DNA and dimerise through a leucine zipper motif [371]. AP-1 transcription factors have diverse functions as the composition of the hetero- or homodimeric complex determines the target genes and transactivation potential. Accordingly, the individual members of AP-1 family can act as oncogenes or tumour suppressors and are often dysregulated in cancer (reviewed in [372, 373]). Notwithstanding, the regulation of cancer cell stemness by AP-1 complexes and the underlying molecular mechanisms have only recently started to come to light.

12.1. Involvement of AP-1 in enhancer reprogramming

Growing body of evidence demonstrates the involvement of AP-1 transcription factors in chromatin remodelling processes that mediate cellular reprogramming and acquisition of stem-like state in cancer. Zhang et al. showed that FOSL1 promotes tumourigenicity and metastasis by establishing super-enhancers at cancer stemness and prometastatic genes. Super-enhancer refers to a large cluster of enhancers with an unusually high levels of transcription factor/complex binding that drives expression of genes controlling cell identity. In cancer, super-enhancers account for the high expression of key oncogenes that maintain the malignant phenotype of tumour cells. Mechanistically,

FOSL1 recruits Mediator complex subunit 1 (MED1) to establish super-enhancers at genomic regions that control expression of protumorigenic genes (e.g., *SNAI2*, *FOSL1*, *CD44*, *EPHA2*, *MCL1*, *BIRC5*, *MMP3* and *MMP8*). Consistently, depletion of FOSL1 leads to disruption of the super-enhancers, inhibition of the key EMT and stemness genes, suppression of migration and self-renewal in vitro, and decreased tumour formation and metastasis in vivo [374]. Bhattacharya et al. demonstrated that JUN is involved in MUC1-C-driven epigenetic reprogramming and dedifferentiation process wherein it interacts with MUC1-C and recruits MUC1-C-activated BAF chromatin remodelling complex to selected genomic proximal enhancer regions. Binding of MUC1-C/JUN/BAF complex leads to an increased deposition of H3K27ac and H3K4me3 epigenetic marks, and enhanced chromatin accessibility on the stemness genes (e.g., *NOTCH*). Silencing of NOTCH inhibits tumoursphere formation [375]. In addition, Bi et al. elucidated the role of JUN in differential binding of oestrogen receptor α (ER α) that facilitates acquisition of therapy resistance through global enhancer and transcription reprogramming. In breast cancer, initial response to oestrogen deprivation therapy is often followed by emergence of resistant subpopulation of cancer cells and subsequent tumour relapse. Mounting data suggest a link between acquired therapy resistance and differential enhancer binding by ER α which is governed by changes in transcription factor-transcription factor and transcription factor-enhancer interactions. Interestingly, JUN is upregulated and interacts with ER α in tamoxifen resistant cells which results in recruitment of ER α to JUN occupied genomic regions and establishment of *de novo* enhancers. Shared binding sites of JUN and ER α exhibit elevated levels of H3K27ac histone mark, and are also bound by BAF and histone acetyltransferase P300. Importantly, ER α occupancy and features associated with active enhancers are lost upon JUN knockdown which confirms the essential role of JUN in global enhancer reprogramming that profoundly alters the breast cancer transcriptional programme. Consequently, the transcriptome changes endow cells with therapy-resistant partial EMT phenotype [376,377].

12.2. AP-1 family members underlie plasticity and therapy resistance

In addition to involvement in establishing a stem-like state, AP-1 family facilitates interconversion between distinct types of glioblastoma stem-like cells [378]. Glioblastoma can be classified into at least three subtypes, namely proneural, mesenchymal and classical, which harbour distinct genetic alterations and show varying responses to therapy [379]. Recent study demonstrates that FOSL1, which is predominantly expressed in the mesenchymal subtype, is upregulated in response to treatment with ionising radiation and promotes resistance to radiation therapy by activating DNA damage response and facilitating acquisition of mesenchymal phenotype [378]. Accordingly, upregulation of FOSL1 induces expression of mesenchymal subtype markers/master regulators (e.g., CD44, C/EBP β , TAZ and phospho-STAT3) and reduces expression of proneural subtype markers/master regulators (e.g., OLIG2 and SOX2) [378,380]. Similarly, members of AP-1 family (c-FOS and c-JUN) are activated by JNK in cervical cancer stem-like cells in response to exposure to UV radiation and their activation promotes radioresistance but the exact mechanism remains to be uncovered [381–383]. Moreover, FOSL1 stimulates transcription of *NANOG*, and induces cell migration, cell invasion and sphere formation in colorectal cancer [384]. In head and neck squamous cell carcinoma, overexpression of c-FOS upregulates the protein levels of *NANOG*, c-MYC, SOX2 and NOTCH1, stimulates EMT and increases sphere formation [385].

In conclusion, the members of AP-1 transcription factor family serve as master regulators that orchestrate cellular reprogramming towards stem-like therapy resistant phenotype. They are key players in phenotypic plasticity as they coordinate global epigenetic and transcriptional changes, for instance during acquisition of partial EMT phenotype.

13. P53

The tumour suppressive transcription factor P53 is the most studied human gene likely due to being the most frequently mutated gene in cancer. It contains two tandem transcription activation domains in the N-terminus followed by a proline-rich domain that contributes to transcription activation. The central region, a site of most cancer-associated mutations, is responsible for DNA binding and is connected to the C-terminal tetramerization domain and unstructured regulatory region through a flexible linker. The classical function of P53 involves its activation in response to cellular stress and subsequent cell cycle arrest followed by DNA repair or apoptosis (reviewed in [386]). In addition to DNA damage response, P53 also controls the balance between self-renewal and differentiation in embryonic stem cells and prevents dedifferentiation of somatic cells, thus acting as a barrier to somatic cell reprogramming. Unsurprisingly, loss-of-function mutation in *P53* gene that disrupts its transcriptional activity correlates with dedifferentiated tumours. Moreover, gain-of-function mutations that cluster in the DNA-binding domain also promote cancer stemness as evidenced by enhanced invasion, resistance to chemotherapy and ability to resist cell death (reviewed in [387]). Recent studies have provided new insights into the mechanisms underlying the stemness-related functions of wild type and mutant P53.

13.1. Role of wild-type P53

The wild-type full-length P53 has been known to induce differentiation of human embryonic stem cells through microRNA networks (reviewed in [387]). More recently, Ren et al. demonstrated that P53 downregulates the expression of mesenchymal markers (ZEB2, N-cadherin, vimentin and fibronectin) and stem cell factors (CD44, OCT4, MYC and KLF4) in prostate cancer through transcriptional activation of miR-145 which has also been implicated in regulation of EMT and stemness in colorectal cancer and Ewing sarcoma [267,388,389]. These molecular changes are accompanied by reduced migration, invasion and sphere formation [390]. In breast cancer, *MYC*, an oncogene whose transcription is directly repressed by P53, becomes overexpressed upon loss of P53 leading to an increased cellular plasticity [391]. In addition to the full-length protein, *P53* gene encodes at least 12 different isoforms through variable promoter usage, alternative splicing and alternative translation start sites yielding proteins with distinct structure and function. Accordingly, the $\Delta 133p53\beta$, a *P53* splice variant lacking the transactivation domain and C-terminal oligomerisation domain but harbouring an additional domain with unknown function, stimulates the expression of *OCT4*, *SOX2* and *NANOG*. The elevated levels of key stemness factors result in enhanced mammosphere formation and metastasis, indicating that the wild-type P53, which is considered to be a tumour suppressor, has the potential to promote stem-like phenotype via alternative protein variants [392].

13.2. Role of mutant P53

The most common genetic alterations affecting *P53* gene are missense mutations in its DNA-binding domain, thus producing a protein with reduced capacity to bind a specific DNA sequence. Along with the loss-of-function mutants, a gain-of-function variants have been identified that promote tumour progression through novel molecular function or novel set of target genes [393]. Loizou et al. reported that *Trp53*^{R172H}, the most common allele in patients with acute myeloid leukemia, enhances self-renewal via upregulation of FOXH1 which in turn drives stem-like transcriptional program by controlling expression of genes associated with differentiation status (e.g., *Runx2* and *Mef2c*) [394]. Moreover, FOXH1 expression facilitates somatic cell reprogramming and is linked to EMT [395–397]. Notwithstanding, the mechanism underlying the regulation of FOXH1 by mutant P53 remains to be determined [394]. A study by Ghatak et al. revealed a novel role of

mutant P53 in non-small cell lung cancer (NSCLC), where it promotes stemness via upregulation of miR-324-5p, a microRNA that targets CUE domain-containing protein 2 (CUEDC2). The consequent CUEDC2 downregulation leads to activation of NF- κ B [398,399].

In conclusion, inactivation of wild-type full-length P53 facilitates establishment of a stem-like phenotype. However, despite being the most-studied human gene, the interplay between stemness and the P53 protein variants that arise from a multilevel regulation awaits to be characterised and understood.

14. NOTCH

The Notch signalling is a highly conserved developmental pathway that orchestrates cell fate decisions. It is activated by an interaction between a transmembrane ligand (DLL1, DLL3, DLL4, JAG1 or JAG2) on one cell and a transmembrane receptor (NOTCH1–4) on a neighbouring cell, therefore being a juxtacrine signalling system that requires cell-cell contact. This ligand-receptor interaction triggers a two-step proteolytic cleavage of the receptor resulting in the release of Notch intracellular domain (NICD) which translocates to the nucleus and promotes transcription in cooperation with the DNA-binding factor RBPJ (also known as CSL), co-activators of the Mastermind-like (MAML) family and co-regulators (reviewed in [400,401]). Importantly, transcriptional targets of Notch signalling pathway are dependent on the cellular context and signal dose explaining its oncogenic or tumour suppressive function in diverse tumour types [400]. Accordingly, mutations in genes encoding NOTCH receptors, most commonly altered components of the pathway, are cancer-specific and range from alterations that allow ligand-independent activation to variants that produce loss-of-function protein (reviewed in [402]). Notch pathway activation through a ligand-receptor complex or a constitutively active variant has been shown to promote self-renewal of stem-like cancer cells (reviewed in [400,403–405]). Recent research has elucidated the downstream signalling cascades whereby NOTCH regulates cell migration, cell proliferation and stem-like cell phenotype in a context-dependent manner.

14.1. NOTCH promotes cell motility and self-renewal but inhibits proliferation

Xiao et al. demonstrated that the expression of Notch ligands JAG1, JAG2, DLL1 and DLL4 as well as Notch receptors NOTCH1 and NOTCH2 is upregulated in stem-like cells of renal cell carcinoma, whereas inhibition of Notch pathway results in loss of stemness features (i.e., self-renewal, migration, invasion, chemoresistance and in vivo tumorigenicity). Mechanistic analysis revealed that NOTCH1 enhances cell migration and invasion through upregulation of CXCR4 and activation of CXCL12/CXCR4 signalling (Fig. 5B) [406]. In addition to facilitating cell motility, CXCL12/CXCR4 axis is involved in promoting self-renewal of stem-like cancer cells as reviewed in [407]. Work by Wang et al. highlighted the role of interaction between JAG1 (expressed by neurons) and NOTCH1 (expressed by glioma cells) in promoting the stem-like phenotype and enhancing the invasion of white matter tracts by glioma cells. The binding of JAG1 to Notch receptor triggers a release of NICD1 which indirectly stimulates expression of SOX2 by activating transcription of SOX9. In turn, upregulation of SOX2 induces TET3-mediated DNA demethylation of NOTCH1 promoter and leads to elevated levels of NOTCH1, thus forming a positive feedback loop [408]. Importantly, different NOTCH receptors can have both redundant and unique functions, and the target genes of a NOTCH receptor are determined by the cellular context [409]. Interestingly, SNAI2 has emerged as a common target of NOTCH receptors in diverse cancer types. In colorectal cancer, constitutively active NOTCH1 enhances stemness and migration by increasing expression of TGF- β pathway effector SMAD3, stem cell marker CD44 and EMT regulator SNAI2 [410]. In triple negative breast cancer, highly expressed and activated NOTCH4 promotes maintenance of the stem-like properties by transcriptionally

upregulating SNAI2 and GAS1. SNAI2 is a core EMT transcription factor that facilitates acquisition of a mesenchymal phenotype. GAS1, short for growth arrest-specific protein 1, downregulates the expression of cyclin D1 and promotes cell cycle arrest in G0/G1-phase. Accordingly, NOTCH4-positive cells are more invasive but less proliferative, the latter contributing to their chemoresistance [411]. In glioblastoma, NOTCH1-induced expression of SNAI2, TAL1 truncated isoform and TAL1 co-factor LMO2 reduces the growth of stem-like glioma cells further supporting the antagonistic effect of Notch pathway activation on cell proliferation. Nevertheless, additional mechanistic studies are required to identify the downstream targets mediating these growth inhibitory effects [412].

14.2. The complexities of NOTCH

Work by Luiken et al. has helped to elucidate the controversial findings on the role of Notch signalling in liver cancer as they showed that Notch function is dependent on the driver gene of the tumour. For instance, NOTCH inhibits MYC-driven carcinogenesis by inducing expression of HES5 which downregulates the pro-proliferative MYC targets *ODC1* and *LDHA*. In contrast, NOTCH promotes AKT-driven tumourigenesis as HES5 interacts with AKT and enhances the stem cell features in a murine model of hepatocarcinogenesis [413]. In addition, Notch signalling plays a key role in establishing phenotypically distinct subpopulations of stem-like cells in colorectal cancer. Mechanistically, NOTCH1 recruits the histone methyltransferase SETDB1 to inhibit Wnt/ β -catenin pathway target genes (e.g., *PROX1*). On the other hand, *PROX1* recruits the nucleosome remodelling and deacetylase (NuRD) complex to NOTCH1 promoter to suppress its expression. This mutual suppression between NOTCH1 and *PROX1* results in two stem-like cell subtypes: 1) LGR5⁺ NOTCH1⁺ *PROX1*[−] BMI⁺ (important during early phases of tumourigenesis) and 2) LGR5⁺ NOTCH1[−] *PROX1*⁺ (highly proliferative, possesses active Wnt signalling, important during progression and metastasis) [414].

In conclusion, the outcome of Notch pathway activation is determined by the cellular context and the activity of other signalling cascades. Nevertheless, reduced proliferation and enhanced migration/invasion are the effects shared by diverse tumour types upon Notch stimulation.

15. STAT

The signal transducer and activator of transcription (STAT) protein family consists of seven members which serve as intracellular effectors of a number of cytokine, growth factor and hormone (e.g., IL-6, insulin, IGF-1, EGF, HGF, PDGF and CSF1) signalling pathways and are primarily activated by non-receptor Janus kinases (JAKs). Activating phosphorylation of STATs stimulates formation of homo- and heterodimers that translocate to nucleus and function as transcription factors. Although mutations in STAT genes are rare, STAT proteins are frequently overactivated in cancer through genetic alterations affecting the components of upstream signalling pathways or negative regulators of STAT. For instance, increased expression and secretion of growth factors or activating mutations in growth factor receptors lead to constitutive activation of STAT proteins. Importantly, STAT3 and STAT5, the most-studied members of the family, exhibit mainly tumour promoting functions, whereas STAT1 can exert either pro-tumourigenic or anti-tumourigenic effects on cancer cells as reviewed in [415–419]. Furthermore, STAT transcription factors have been implicated in regulation of stemness, EMT and therapy resistance (reviewed in [420–422]).

15.1. The regulation of pluripotency factors and microRNAs by STAT

Tumour microenvironment serves as a niche for stem-like cancer cells and its components play a key role in supporting stemness state by

activating intracellular signal transduction pathways in tumour cells (reviewed in [423,424]). Kryczek et al. have shown that CD4 T cell-produced IL-22 promotes stemness via activation of STAT3 and subsequent upregulation of core stem cell genes *NANOG*, *SOX2* and *POU5F1*. Mechanistically, STAT3 stimulates transcription of *DOT1L*, a histone H3K79 methyltransferase which in turn mediates dimethylation of H3K79 (H3K79me₂, an active histone mark) on the proximal promoter regions of *NANOG*, *SOX2* and *POU5F1* [425]. Similarly, Wang et al. demonstrated that IL-6 stimulation results in STAT3 activation and elevated levels of *SOX2*, *NANOG* and *FOSL1*. Importantly, *FOSL1* is required for IL-6-induced cell migration, cell invasion, sphere formation and chemoresistance. STAT3 positively affects the interaction between *FOSL1* and HDAC6 which leads to the deacetylation of *FOSL1* at the K116 residue located within its DNA-binding domain. The deacetylation of K116 increases transcriptional activity of *FOSL1* and promotes its binding to the *NANOG* promoter [384]. Furthermore, IL-6-activated Stat3 pathway also enhances stemness in breast cancer by stimulating expression of *Sox2*, *Oct3/4* and *Nanog* [426]. An independent study by Wu et al. described the role of long non-coding RNA (lncRNA) *HLA-F antisense RNA 1* (*HLA-F-AS1*) in STAT3-induced cell proliferation and stemness in triple negative breast cancer. *HLA-F-AS1* becomes upregulated due to the activation of STAT3 which directly interacts with *HLA-F-AS1* promoter region and drives transcription. *HLA-F-AS1* promotes cell proliferation, enhances sphere formation and inhibits apoptosis by sponging miR-541–30 which would otherwise bind to the mRNA of *TRABD* and trigger its degradation. The mechanism downstream of *TRABD* remains to be uncovered, but the rescue experiments confirmed that upregulation of *TRABD* reverses the suppression of cell proliferation and stemness, and promotion of cell cycle arrest and apoptosis by *HLA-F-AS1* silencing [427]. Work by Chen et al. further supported the interplay between STAT signalling and miRNA network by showing that STAT3 enhances self-renewal, sphere formation, chemoresistance and tumorigenicity through upregulation of miR-92a. This leads to an activation of the Wnt pathway through the silencing of *DKK1*, a Wnt antagonist and miR-92a target [428].

15.2. STAT promotes fatty acid β -oxidation

Lipid metabolism plays an integral role in tumorigenesis as fatty acids are the key building blocks of biological membranes, can function as secondary messengers in signalling pathways and serve as a form of energy storage [429]. Fatty acid β -oxidation (FAO) is a catabolic process by which fatty acids are broken down to produce energy. The utilisation of FAO provides tumour cells with a metabolic advantage as it allows synthesis of approximately six times more ATP than oxidation of carbohydrates, and also generates NADH and FADH₂ which are essential for detoxification of reactive oxygen species (ROS) [430]. Recent evidence demonstrates that FAO is essential for the self-renewal and chemoresistance of stem-like cells in breast cancer. Importantly, JAK/STAT3 signalling activates FAO through transcriptional upregulation of carnitine palmitoyltransferase 1B (CPT1B), a rate-limiting enzyme for FAO, which is required for mitochondrial uptake of lipids (Fig. 5H). Knockdown of STAT3 or inhibition of CPT1 by etomoxir significantly decreased ATP levels and cell viability of stem-like cancer cells but had only a minimal effect on the differentiated tumour cells. Furthermore, inhibition of FAO also results in reduced sphere formation capacity. Interestingly, exogenous lipid supplies serve as oxidation substrates for JAK/STAT3 regulated FAO implying the role of adipocytes in supporting stemness state in cancer [431].

15.3. STAT3 controls cell cycle, DNA damage repair and EMT

Tumoursphere formation assay is an in vitro approach used for evaluating the percentage of stem-like cancer cells within a given population or sample by assessing self-renewal capacity [432]. In order to successfully form spheres through several generations, cell must be able

to proliferate and maintain stemness phenotype whilst undergoing cell division cycle. In addition to the above-mentioned examples describing the upregulation of stem cell genes, STAT3 also controls the expression of cell cycle regulators. In medulloblastoma, STAT3 promotes cell cycle progression by inducing expression of *MYC* and *CCND1* (Fig. 2A) [433]. In ovarian cancer, STAT3 knockout cells exhibit a reduced expression of cyclins (e.g., *CCNA1*, *CCNA2*, *CCNB1*, *CCNB2*, *CCND*, *CCNE1*, *CCNE2* and *CCNF*) and cyclin-dependent kinases (CDK1 and CDK2) which manifests in cell cycle arrest [434]. In colorectal cancer, STAT3-induced expression of *CCND2* is essential for the intact cell cycle, stemness and radioresistance. *CCND2* silencing leads to a downregulation of cell cycle genes involved in G1 (*MYCN*, *JUN* and *MYC*), G1/S (*CCNE2*, *E2F1*, *MYBL2*, *MYB* and *TFDP1*) and S/G2 (*CDC20*, *AURKB*, *CKS1* and *CKS2*), diminishes levels of genes participating in replication of DNA (*DUT*, *RRM1*, *TYMS*, *MCM2*, *MCM4* and *MCM7*), and reduces expression of genes facilitating DNA repair (*UNG1*, *FEN1*, *PRKDC*, *MSH2* and *RAD54L*). Collectively, upregulation of *CCND2* allows persistent proliferation of stem-like cancer cells without accumulation of DNA damage following radiation therapy [435]. In addition, STAT3 promotes EMT through induction of *TWIST* and *SNAIL* expression, making it a truly multifunctional transcription factor controlling stemness and stemness-associated features [433,434]. Lastly, STAT1 has also been demonstrated to enhance the stemness of cancer cells but the underlying molecular mechanisms are less studied [436,437].

In conclusion, STAT3, activated by autocrine and paracrine signalling, is an integral player in the stemness network shaping the epigenetic profile, transcriptionally activating stem cell genes, mediating rewiring of cellular metabolism, stimulating cell proliferation and inducing EMT.

16. GLI

The GLI proteins, first identified through gene amplification and high expression in glioma, are zinc-finger transcription factors that serve as main effectors of Hedgehog pathway [438]. All three family members, namely GLI1, GLI2 and GLI3, harbour five Kruppel type zinc-finger motifs that facilitate specific and stable interaction with DNA. A nuclear localisation signal (NLS) and nuclear export signal (NES) allow nucleocytoplasmic shuttling while transactivation domain and trans-repression domain participate in regulation of gene expression. GLI2 and GLI3 possess a C-terminal transactivation and N-terminal repressor domains, whereas the only regulatory region of GLI1 is the transactivation domain in C-terminus (reviewed in [439]). Despite the potential to function as a multifaceted regulators, GLI3 acts mainly as a strong repressor and full-length GLI2 serves as a weak activator [440–444]. GLI1 has been reported as a transcriptional activator in most settings. GLI transcription factors are aberrantly expressed and/or activated in many tumours as a result of gene amplification, overproduction of Hedgehog ligands, mutations in the components of Hedgehog pathway or activation via non-canonical route (e.g., *RAS*/*RAF*/*MEK*/*ERK*, *PI3K*/*AKT*/*mTOR* and *TGF- β* pathways)(reviewed in [439]). Upon activation, GLI proteins regulate genes involved in proliferation, EMT, cell survival, drug resistance and stemness (reviewed in [439,445]).

16.1. The interplay between GLIs, stemness factors and signalling pathways

Importantly, each of the three family members has been reported to support acquisition and/or maintenance of stem-like state in studies focusing on diverse tumour types. In stem-like cells of lung adenocarcinoma, GLI1 is transcriptionally upregulated by *PAX6* and promotes stemness by stimulating expression of *SOX2* which thereafter enhances the levels of stem cell factors *OCT4* and *NANOG* while suppressing the transcription of lineage specifying factors *HOPX* and *NKX2-1*. In the differentiated cancer cells, *PAX6* gene is silenced by promoter methylation. Decreased sphere formation capacity and increased expression of differentiation markers following GLI1 knockdown supports the role of

GLI1 in driving cancer cells toward a stem-like state [446]. Furthermore, Po et al. revealed a self-amplifying autocrine signalling loop that involves a non-canonical activation of GLI1 through MAPK/ERK signalling pathway. Among other ligand-receptor pairs, MAPK/ERK pathway is activated by VEGFA binding to its receptor NRP2 which leads to a phosphorylation and nuclear localisation of GLI1. Importantly, VEGFA and NRP2 are direct targets of GLI1, thus completing self-reinforcing feedback loop which facilitates maintenance of stemness phenotype [447]. In addition to lung adenocarcinoma, GLI1 together with GLI3 also enhance stem-like features in liver cancer as their knockdown results in reduced SOX2 expression, sphere formation capacity and in vivo tumorigenicity [448]. Liu et al. work on hepatoblastoma, a most common liver tumour in early childhood, uncovered a mechanism whereby GLI2 facilitates cell proliferation, migration, invasion and stemness through transcriptional activation of circular RNA circ-STAT3 (circ_0043800) which is formed of STAT3 pre-mRNA exons 14 and 24 via back-splicing. Circ-STAT3 locates in the nucleus and serves as a competing endogenous RNA (ceRNA) by sponging miR-29a/b/c-3p which targets STAT3 and GLI2. Accordingly, GLI2-induced expression of circ-STAT3 leads to an upregulation of STAT3 and GLI2, whereas knockdown of circ-STAT3 results in decreased levels of SOX2, OCT4 and NANOG, and impairs cell proliferation, migration, invasion and sphere formation [449]. Along with the full-length GLI2 that functions as a weak activator, the N-terminal repressive fragment of GLI2 has also been implicated in stemness of cancer cells. The N-terminal fragment is generated by the ubiquitin-mediated degradation of C-terminus and downregulates the expression of SOX17 in colorectal cancer endowing the cells with enhanced sphere formation capacity through subsequent upregulation of PAX6 (Fig. 6C) [450–452]. Earlier studies have shown that SOX17 negatively regulates the activity of Wnt signalling pathway and silencing of SOX17 through promoter hypermethylation is an early event in colorectal cancer [6]. Interestingly, β -catenin, a Wnt pathway transcriptional effector, is able to bind to the promoter region and activate transcription of PAX6, however, this finding is yet to be confirmed in the context of colorectal cancer [453]. Furthermore, expression of PAX6 enhances cell proliferation, invasion and colony formation supporting the potential role of GLI2/SOX17/ β -catenin/PAX6 axis in promoting stemness in colorectal cancer [454]. Volnitskiy et al. further elucidated the underlying molecular mechanisms by showing that in glioma, expression of stemness markers *FOXM1*, *BMI1*, *SOX2* and *OCT4* decreases upon downregulation of GLI1, GLI2 or GLI3. Notably, inhibition of GLI1 and GLI2 reduces the levels of TET1, a methylcytosine dioxygenase facilitating DNA demethylation, suggesting their potential role in mediating epigenetic reprogramming [455].

16.2. GLIs promote survival and enhance EMT

In addition to the regulation of stemness and in vivo tumorigenicity, several studies have reported increased cell death upon knockdown or inhibition of GLI1, GLI2 or GLI3 implying their involvement in cell survival networks [455]. GANT61 is a Hedgehog pathway inhibitor whose binding to GLI1 and GLI2 blocks their transcriptional activity. Treatment with GANT61 induces apoptosis in lung adenocarcinoma and prostate cancer as evidence by the decreased expression of BCL2, and enhanced cleavage of caspase 3 and PARP [456,457]. Moreover, inhibition of GLI1 and GLI2 reduces the expression of mesenchymal marker vimentin and diminishes invasiveness of prostate cancer cells [458]. *Gli1* has been demonstrated to bind the promoter and promote transcription of EMT master regulators *Snail1*, *Zeb1* and *Twist1* [459]. Altogether, regulation of stemness, cell survival and EMT, coupled with the upregulation of ABCG2 and ABCB1 (efflux pumps responsible for multidrug resistance), make GLIs pivotal players controlling the most pernicious features of tumour cells [460–462].

In conclusion, considering that majority of oncogenic pathways can activate GLI transcription factors, they can potentially serve as a node of stemness/plasticity network integrating upstream signals and

coordinating downstream responses enabling adaptation to environmental cues. Despite the growing body of evidence starting to uncover the molecular mechanisms employed by GLIs to promote stemness and control stemness-associated features, more studies are warranted to better understand the function of individual family members in different tumour types.

17. ETS

The ETS (E26 transformation specific or erythroblast transformation specific or E-twenty-six) is a family of 28 transcription factors that all contain a highly conserved DNA-binding ETS domain (reviewed in [463, 464]). They govern a large number of processes, including differentiation, cell division cycle, migration, apoptosis and angiogenesis. The ETS proteins are aberrantly activated in cancer through diverse mechanisms, such as chromosomal rearrangement, gene amplification, gain-of-function mutation, increased stability and *cis*-acting mutation. Importantly, ETS activity is commonly associated with enhanced self-renewal in solid tumours (reviewed in [465]).

Pezze et al. have shown that expression of transcriptional repressor ETV7 increases the percentage of CD44⁺/CD24^{low} breast cancer stem-like cells along with enhancing mammosphere formation capacity, and resistance to chemo- and radiotherapy. Mechanistically, ETV7 inhibits transcription of interferon response genes (e.g., IFITM2, IFI35, PROCR and HERC6), whereas treatment with IFN- β , which restores interferon signalling, reduces the stemness of breast cancer cells [466]. This is in accordance with the previous studies that have described basal repression of interferon response signature in stem-like cancer cells, and reduced self-renewal and tumorigenic capacity upon treatment with IFN- β as reviewed in [467]. In addition, ETS family member ELK-1 promotes self-renewal of brain tumour initiating cells by activating transcription of *SOX2* [468]. Lastly, epithelium-specific ETS transcription factor 1 (ESE-1) inhibits expression of NANOG in human embryonic carcinoma cells which results in reduced colony formation capacity [469].

Altogether, the ETS protein family comprises a diverse set of transcription factors that are potentially involved in the regulation of cancer cell stemness but further studies are necessary to characterise the mode of action, identify the target genes and uncover the functional relevance.

18. BRACHYURY

The T-box transcription factor BRACHYURY is a developmental regulator that is involved in the formation of primitive streak and mesoderm during early embryonic development. It is usually not expressed in normal adult tissue but is detected in various human cancers, where it promotes tumour cell migration, invasion, and metastasis [470]. The molecular mechanisms underpinning the regulation of cancer cell stemness by BRACHYURY have only recently started to come to light.

BRACHYURY expression may slow cell cycle progression, enabling tumour cells to become less susceptible to chemotherapy and radiation in human carcinomas [471]. BRACHYURY has also been shown to regulate EMT and stem cell markers such as NODAL, LEFTY, OCT4, PAX6, REX1 and NANOG in adenoid cystic carcinoma cells [472]. BRACHYURY itself is regulated by β -catenin and in turn regulates NANOG and other stemness markers in mesenchymal-like colorectal cancer stem-like cells to impose a plastic state [473]. BRACHYURY is a crucial regulator of stemness in chordoma by controlling the synthesis and stability of yes-associated protein (YAP), a key regulator of tissue growth and homeostasis. The BRACHYURY/YAP regulatory pathway is associated with tumour stemness and aggressiveness not only in chordoma but also in more common aggressive cancers such as lung cancer and glioblastoma [474]. Although the role of BRACHYURY in glioblastoma is contradictory, this factor could still serve as a potential therapeutic target in patients who do not respond to conventional

chemotherapeutic drugs [475]. Intriguingly, expression of *BRACHYURY* induces a mesenchymal state and reduces sensitivity of human carcinoma cells to immunotherapy. High levels of *BRACHYURY* repress cyclin-dependent kinase inhibitor 1 (p21) and this results in decreased susceptibility to immune-mediated lysis by antigen-specific CD8⁺ T cells, innate natural killer (NK) cells and lymphokine-activated killer (LAK) cells [476]. Lastly, forced co-expression of *BRACHYURY* and *SOX2* strongly upregulate EMT and stem cell markers including fibronectin, *SNAI1*, *SNAI2*, *ZEB1*, and *TGF-β2*, and the self-renewal phenotype suggests that *BRACHYURY* and *SOX2* synergistically promote cancer stemness in oral cancer cells [477].

Altogether, recent findings indicate that *BRACHYURY* has an important function in promoting EMT and stem-like characteristics in various aggressive malignancies.

19. HOX

First identified in *Drosophila*, homeobox (HOX) genes are highly conserved determinants of body patterning in embryonic development. HOX genes are expressed in a tightly controlled spatial-temporal manner whereby the chromosomal position of the HOX gene corresponds to the timing and location of expression in the developing embryo. This mechanism is conserved from *Drosophila* to human [478,479] (reviewed in [480]). Mammals have 39 HOX genes grouped into four clusters of 9–11 genes, each made up of two exons the second of which encodes the 180-nucleotide homeobox containing the DNA-binding homeodomain [480]. HOX transcription factors regulate the expression of genes involved in all stages of cellular differentiation and are therefore important for both stem and differentiated cell functions. HOX genes also have a hierarchical expression in adult tissues whereby particular HOX genes are expressed in stem cells, progenitors or differentiated cells to maintain cellular identity [481].

Many HOX genes are dysregulated in cancer with certain clusters perturbed in a tissue-specific manner (i.e., *HOXA* in breast, *HOXB* in colon, *HOXC* in prostate and lung, and *HOXD* in colon and breast) [482–484]. Interestingly, the epigenetic upregulation of HOX genes observed in glioma appears to be reminiscent of that observed during development albeit without the controlled pattern of expression seen in embryogenesis, suggesting the reactivation of some stem cell-associated functions [485]. Indeed, in many cancers it appears to follow that those HOX genes important for stem cell function in normal development or adult tissues are cancer-promoting, whereas those that are expressed in more differentiated cells have tumour-suppressive functions. For example, *HOXA9* and *HOXB4* are important for both normal intestinal and haematopoietic stem cells and both also promote stemness in colorectal cancer and acute myeloid leukaemia (AML), respectively [486–489]. This correlation may be tissue-dependent as *HOXB4* inhibits tumorigenesis in cervical cancer, suggestive of an anti-stemness function [490]. Another example of a tissue-dependent function is *HOXA5* which marks stem-like cells in glioma but maintains the differentiated state in breast cancer cells [491,492]. *HOXA1* is also a negative regulator of stemness in cancer cell lines and functions by preventing the transcription of *NANOG*. Epigenetic silencing of *HOXA1* via the lncRNA *HOTAIR* results in activation of *NANOG* expression and an increase in stem-like properties [493]. HOX genes promote the stemness phenotype by a variety of molecular mechanisms including those which occur during embryogenesis and EMT such as FGF, *TGF-β* and Wnt signalling [494,495]. For example, *HOXB7* and *HOXA13* both activate EMT via the *TGF-β* pathway in breast cancer and glioma, respectively. Both also promote the expression of other transcription factors including *β*-catenin, *c-MYC* and *SNAI2* [495,496]. Furthermore, *HOXB7* has also been shown to promote stemness via direct transcriptional activation of *Lin28B* which in turn promotes the expression of *SOX2* [497].

These studies provide strong evidence that stem cell-related HOX genes also regulate stemness in a cancer or tissue-dependent manner. It is likely that this regulation occurs via multiple pathways which

converge to promote the stem-like phenotype. The highly specific role of certain HOX genes for normal stem cell and cancer stem-like cell function suggest they may be of use as therapeutic targets or stemness markers.

20. FOX

Like HOX genes, the Forkhead-Box (FOX) family of transcription factors was originally identified in *Drosophila* and is a highly conserved gene family with multiple biological functions including the regulation of pluripotency, development, metabolism and cell cycle [498,499] (reviewed in [500,501]). Humans have 44 FOX genes categorised into 19 subclasses (A–S) based on sequence similarity in the Forkhead-Box (FHB) DNA-binding domain. Each subclass of FOX proteins has specific functions, e.g., FOXA factors are involved in development and differentiation, whereas FOXO factors are important for metabolism. Certain specific factors also have well-defined roles, for instance, FOXM1 in cell cycle control and FOXF in the development of speech (reviewed in [500]). In accordance with this wide functional range, FOX transcription factors can act as both tumour promoters or suppressors depending on the factor in question. Certain FOX factors are important for normal stem cell function and induced pluripotency and therefore may also regulate stem-like cells in cancer [502,503].

20.1. FOXO subfamily

The FOXO family consists of four members, FOXO1, FOXO3, FOXO4 and FOXO6, which are all negatively-regulated by insulin-like growth factor (IGF) or insulin-induced PI3K signalling. Activation of the PI3K pathway results in phosphorylation of protein kinase B (PKB) which impairs the nuclear translocation of FOXO factors preventing FOXO-mediated transcription of diverse gene targets including those involved in metabolism (e.g., *G6PC1* and *PCK1*) and the cell cycle (e.g., *CDKN1B*) [504,505]. In accordance with the overactivation of the IGF/Insulin-PI3K axis in both diabetes and cancer, FOXO transcription factors usually function as tumour suppressors and inhibitors of stemness properties in many tumour types [506–510]. FOXOs may exert a tumour suppressive function by inducing cell cycle arrest via the upregulation of cell-cycle inhibitors (e.g., p15 and p27) and downregulation of cyclin D1 to block S-phase entry [504]. However, FOXO3 has been found to be tumour promoting in breast and colorectal cancer, and increases the frequency of stem-like cells in chronic myeloid leukaemia (CML) [511–513]. These apparently conflicting reports suggest that the effects of FOXO factors on stem-like cell function may be factor, stage or tissue-dependent.

20.2. Roles of other FOX family members

The FOXM1 protein is a well-characterised mediator of the cell cycle in adult tissues where it regulates both the transition from G1 to S-phase and mitosis [514]. FOXM1 has also been shown to promote expression of the pluripotency genes *OCT4*, *NANOG* and *SOX2* [515]. In addition, FOXM1 interacts with many pathways implicated in cancer progression such as RAS/RAF/MAPK, Hippo/YAP, Hedgehog and PI3K/AKT, and is also associated with tumourigenic mutations including activation of KRAS and loss of p53 [516–522]. Due to these roles, FOXM1 is associated with highly proliferative cells in developing tissues and functions as an oncogene in cancer [516]. FOXM1 has been found to promote stemness in many cancers, including pancreatic ductal adenocarcinoma, glioblastoma multiforme, hepatocarcinoma and breast cancer [523–526]. Many other FOX proteins also regulate stemness, including FOXQ1 which promotes cancer stem-like cell function in colorectal cancer via sirtuin 1 (SIRT1)-mediated nuclear translocation of *β*-catenin, and FOXC1 which promotes stemness features in NSCLC via direct transcriptional activation of *β*-catenin [527,528]. FOXD1 promotes cancer stem-like cells in oral squamous carcinoma cells via direct

Table 1
Selected examples of transcription factor deregulation in cancer.

Family	Factor	Tumour type	Mechanism	Result	Reference
SOX	SOX2	Gastric	Enhanced mRNA stability	Upregulation	[1]
	SOX2	Endometrial	Enhanced mRNA stability	Upregulation	[2]
	SOX2	Lung	Gene amplification	Upregulation	[3]
	SOX2	Pancreas	Downregulation of miR-1181	Upregulation	[4]
	SOX9	Colon	Epigenetic derepression	Upregulation	[5]
MYC	SOX17	Colon	Promoter hypermethylation	Downregulation	[6]
	MYC	Leukaemia	Gene amplification	Upregulation	[7]
	MYC	Leukaemia	Enhanced translation	Upregulation	[8]
	MYC	Various	Enhancer amplification	Upregulation	[9]
	KLF4	Liver	miR-10b-mediated silencing	Downregulation	[10]
KLF	KLF5	Various	Enhancer amplification	Upregulation	[9]
	OCT4	Lung	Histone acetylation	Upregulation	[11]
OCT4	OCT4	Breast	Activation of NF- κ B pathway	Upregulation	[12]
	NANOG	Liver	Transcriptional regulation by STAT3	Upregulation	[13]
NANOG	NANOG	Breast	Enhanced mRNA stability	Upregulation	[14]
	NANOG	Glioma	Downregulation of miR-134	Upregulation	[15]
HIF	HIF-1/2 α	Kidney	Loss of pVHL	Upregulation	[16]
	HIF-1 α	Breast	Post-transcriptional regulation by MYC	Upregulation	[17]
EMT TFs	ZEB1	Breast	Transcriptional regulation by RAE1	Upregulation	[18]
	SNAIL	Liver	Enhanced translation	Upregulation	[19]
SMAD	TWIST	Breast	Transcriptional regulation by STAT3	Upregulation	[20]
	SMAD3	Breast	EZH2-mediated protein methylation	Activation	[21]
β -catenin	SMAD4	Pancreatic	Homozygous deletion	Inactivation	[22]
	β -catenin	Colon	Mutation in <i>CTNNB1</i> gene	Upregulation	[23]
AP-1	β -catenin	Liver	Mutation in <i>CTNNB1</i> gene	Upregulation	[24]
	FOSL1	Pancreatic	Loss of SMAD4	Upregulation	[25]
P53	c-JUN	Glioma	Enhanced translation	Upregulation	[26]
	P53	Various	Missense mutation	Inactivation	[27]
NOTCH	NOTCH1	Lung	Loss of inhibitory protein NUMB	Upregulation	[28]
	NOTCH1	Leukaemia	Mutation	Activation	[29]
STAT	STAT3	Lung	Autocrine IL-6 signalling	Upregulation	[30]
	STAT3	Leukaemia	Missense mutation	Activation	[31]
GLI	GLI1	Lymphoma	Transcriptional regulation by MYC	Upregulation	[32]
	GLI1	Glioma	Gene amplification	Upregulation	[33]
ETS	GLI1	Liver	Promoter demethylation	Upregulation	[34]
	GLI2	Gastric	Downregulation of miR-144–3p	Upregulation	[35]
BRACHYURY	ETV1	Prostate	Chromosomal rearrangement	Upregulation	[36]
HOX	BRACHYURY	Chordoma	Gene duplication	Upregulation	[37]
	HOXA4	Leukaemia	Histone methylation	Upregulation	[38]
FOX	HOXA9	Leukaemia	Chromosome translocation	Upregulation	[39]
	FOXM1	Liver	Constitutively active RAS	Upregulation	[40]
RUNX	FOXO3	Neuroblastoma	Activation of PI3K/AKT signalling	Inactivation	[41]
	RUNX1	Leukaemia	Missense or nonsense mutation	Inactivation	[42,43]
SALL	RUNX3	Breast	Promoter hypermethylation	Downregulation	[43]
	SALL4	Lung	Activation of EGFR signalling	Upregulation	[44]
NF- κ B	Various	Various	Inflammatory microenvironment	Activation	[45]

CTNNB1, catenin beta 1 (β -catenin); EGFR, epidermal growth factor receptor; EZH2, enhancer of zeste homolog 2; IL-6, interleukin 6; miR, microRNA; PFN2, profilin 2; PI3K, phosphoinositide 3-kinase; pVHL, von Hippel-Lindau protein; RAE1, ribonucleic acid export 1; SMAD4, mothers against decapentaplegic homolog 4.

transcription of SNAIL2, resulting in EMT and increased stem-associated properties such as migration and invasion [529].

In conclusion, further studies are required to characterise all the members of this highly complex gene family and to determine their relevance to cancer stem-like cell function. Of all the FOX transcription factors, FOXM1 appears to have the strongest and most consistent evidence to-date for stemness-promotion and may therefore be a useful stem-like cell marker or therapeutic target.

21. RUNX

RUNX (runt-related transcription factor) genes are a small family of 3 transcription factors (RUNX1–3) that can activate or inhibit gene expression in a variety of processes. All RUNX proteins have an N-terminal Runt DNA-binding domain which has weak affinity for DNA and therefore requires the CBF β subunit as a binding partner to strengthen transcriptional activity [530,531]. RUNX factors are important for the function of normal stem cells in hematopoietic, neural, muscle and mammary gland tissues. They have also been implicated in cancers of these tissues with both tumour suppressive and onco-supportive functions [530,532]. RUNX factors have multiple target genes including

CDKN1A, *TGFBR1* and *CCND3*, and can therefore modulate many downstream pathways involved in cancer such as p53, TGF- β signalling and cell cycle control [533].

21.1. RUNX family in haematological malignancies

RUNX1 is essential for the endothelial-to-hematopoietic cell transition which occurs during development and for hematopoietic stem cell function in adult tissues [532,534,535]. RUNX1 is highly significant in haematological malignancies and a frequent site of chromosomal translocation in leukaemia. Mutations in RUNX1 can be both activating or inhibiting and therefore it may function as either a tumour suppressor or oncogene depending on the molecular context [530]. RUNX1 can promote stem cell function in both normal hematopoietic stem cells and in leukaemia-initiating cells via transcription of the *PU.1* gene. In both cases, RUNX1 binding to the *PU.1* locus promotes a formation of chromatin loop between the enhancer and promoter to activate *PU.1* gene transcription (Fig. 3D). *PU.1* (also known as *SPI1*) belongs to the ETS transcription factor family and regulates the expression of genes involved in haematopoietic cell identity [534,535].

21.2. RUNX family in breast cancer

RUNX proteins are also important in mammary gland development and breast cancer with RUNX factors 1–3 each having specific roles. In the development of the mammary gland, RUNX1 promotes a luminal cell fate via E2F suggesting that it is important for differentiation [536]. In contrast, RUNX2 may have a stem-cell related function as RUNX2 deletion impaired mammary gland regeneration and overexpression inhibited alveolar differentiation during pregnancy [537,538]. RUNX3 suppresses oestrogen receptor signalling which is a central regulator of the mammary gland and breast cancer development (reviewed in [539]). As would be expected, RUNX1 and RUNX3 therefore usually act as tumour suppressors in breast cancer whereas RUNX 2 is tumour-promoting. Furthermore, RUNX2 expression was elevated in CD24-negative cancer stem-like cells whereas RUNX1 overexpression decreased stemness [540,541]. Mechanistically, RUNX1 exerts antagonistic effect on EMT by directly inhibiting transcription of *ZEB1* while both RUNX1 and RUNX3 attenuate YAP-mediated cell migration and sphere formation by forming an inhibitory complex with YAP [541, 542]. However, RUNX functions may differ between breast cancer subtypes. RUNX1 appears to act as a tumour suppressor in luminal breast cancer but high RUNX1 expression in basal breast cancer is associated with poor prognosis [543]. The role of RUNX1 in cancer is comprehensively reviewed in [544].

Conflicting information regarding the role of RUNX factors in cancer stem-like cells shows that further studies are required to determine their cancer and tumour subtype-specific functions.

22. SALL

Sal-like proteins (SALL) are a family of transcription factors consisting of SALL1–4 that play critical roles in organogenesis during embryonic development [545]. The best characterised family member SALL4 is a stem cell transcription factor that regulates the pluripotency of embryonic stem cells along with NANOG, OCT4, SOX2 and SMAD2/3. SALL4 forms a core transcriptional network with OCT4, NANOG, and SOX2 to concurrently activate self-renewal and repress differentiation-related genes in mouse embryonic stem cells [546]. Like other stem cell factors, it is not expressed in most adult tissues but reactivated in various malignancies, including lung cancer, hepatocellular carcinoma, breast cancer, gastric cancer, colorectal cancer, osteosarcoma, acute myeloid leukaemia, ovarian cancer and glioma. SALL4 overexpression promotes proliferation, invasion, and migration in cancers through activation of the Wnt/ β -catenin, PI3K/AKT and Notch signalling pathways [547].

SALL4 promotes the stemness of cancer cells in many tumour types and it is generally associated with poor prognosis. Recently, it was found that SALL4 enhances the stemness of basal-like breast cancer cells by the upregulation of KHDRBS3, a splicing factor for CD44 [548]. SALL4 protein is stabilized by THG-1 and this induces stemness genes NANOG and OCT4, and promotes tumoursphere growth of oesophageal squamous cell carcinoma cells [549]. The expression of SALL4 in lung cancer cells is induced by the activation of EGFR/ERK1/2 signalling pathway while the knockdown of SALL4 expression suppresses spheroid formation, invasion, metastasis and chemoresistance, and downregulates the expression of cancer stem cell marker CD44 [550]. Similarly, silencing of SALL4 in glioma cells induces cell cycle arrest, enhances early apoptosis, inhibits invasion and increases sensitivity to chemotherapeutic agents [551].

Altogether, SALL4 supports the stem cell-like characteristics of cancer cells in a range of tumour types.

23. NF- κ B

The nuclear factor kappa-light-chain-enhancer of activated B-cells (NF- κ B) transcription factor family comprises five members, namely p65 (RelA), RelB, c-Rel, NF- κ B1 (p105/p50), and NF- κ B2 (p100/p52) which

share a conserved N-terminal Rel homology domain, and form homo- and heterodimers to regulate transcription. The NF- κ B signalling can be activated via a canonical or non-canonical pathway in response to variety of stimuli, including viral or bacterial infection, exposure to pro-inflammatory cytokines, mitogens and growth factors, necrotic cell products, DNA damage, and to oxidative stress conditions [552]. In turn, NF- κ B controls cancer cell proliferation and survival, EMT, angiogenesis, cellular metabolism, therapy resistance and cancer stem-like cell formation [553]. Therefore, the NF- κ B transcription factor family connects inflammation to tumourigenesis [552]. Taking into consideration the essential role of inflammation in tissue regeneration and repair, the stemness enhancing function of NF- κ B pathway activation is unsurprising [553].

The NF- κ B pathway is constitutively activated preferentially in the cancer stem-like cells of diverse malignancies as reviewed in [554–557]. In lung cancer, inhibition of NF- κ B signalling reduces stemness, self-renewal and migration capacity of cancer stem-like cells together with significantly decreasing the expression of genes involved in the EMT (SNAIL and TWIST) and apoptosis resistance (BCL-2 and BIRC5) (Fig. 4D). This suggests that inhibition of NF- κ B is sufficient to prevent the EMT and induce apoptosis in lung cancer stem-like cells [558]. The NF- κ B signalling has been implicated in maintenance of telomere length which supports cell division. Telomeres can be extended by either the telomerase complex or the alternative lengthening of telomeres (ALT) pathway. Robinson et al. reported that activation of the transcription factor NF- κ B promotes Notch signalling and the institution of ALT by transcriptional stimulation of *JAG1* [559]. Hence, this mechanism could be targeted therapeutically in ALT-driven cancers and in cancer cells that develop resistance to anti-telomerase therapies. In hepatocellular carcinoma, the inflammatory microenvironment promotes stemness properties and metastatic potential of cancer cells via the NF- κ B/miR-497/SALL4 axis [560].

In conclusion, activity of NF- κ B transcription factors underlies the inflammation-mediated self-renewal and metastasis phenotypes which suggests that this signalling axis could serve as a target for anticancer strategies.

24. Clinical relevance

24.1. Mechanisms of transcription factor deregulation

The aberrantly expressed or activated transcription factors underlie the malignant gene expression programmes that endow cancer cells with stem-like properties. The transcription factor expression and/or activity can be altered by the following mechanisms: 1) genetic alteration (amplification, deletion, point mutation, translocation or inversion), 2) epigenetic modification, 3) transcriptional regulation, 4) post-transcriptional regulation (RNA modification and RNA interference), and 5) post-translational regulation (protein modification and proteolysis) (Table 1).

First, a change in the copy number or DNA sequence of a gene or a genomic regulatory element can affect both the expression and function of the associated transcription factor. For example, the chromosome segment encompassing *SOX2* gene is amplified in 23–27% of lung cancer samples and the amplification of *GLI1* has been detected in 4% of gliomas [3,561,562,33]. In contrast, SMAD4, a transcription factor that predominantly functions as a tumour suppressor, is inactivated in 55% of pancreatic cancers by homozygous deletion or intragenic mutation [22]. In acute myeloid leukaemia, missense or nonsense mutation in the DNA-binding domain of RUNX1 disrupts its binding to DNA, thereby inactivating its function [42,563,564]. Additionally, genome-wide studies have revealed that disease-associated somatic variants are concentrated in non-coding regions of the genome [565]. More recently, Zhang et al. showed that genomic amplification of regulatory regions that harbour super-enhancers drives overexpression of *KLF5* and *MYC* in multiple cancer types [9].

Second, the expression of a transcription factor is also regulated/deregulated by epigenetic mechanisms which include DNA methylation and histone modification. The cancer cells are characterised by aberrant DNA methylome (i.e., site-specific hypermethylation and genome-wide hypomethylation) which has been shown to mediate silencing of tumour suppressors and facilitate activation of oncogenes (reviewed in [566]). For instance, demethylation of *GLI1* promoter in hepatocellular carcinoma leads to upregulation of *GLI1* expression accompanied by dedifferentiation and acquisition of progenitor-like features [34]. In colorectal cancer, *SOX17* expression is downregulated through promoter hypermethylation [6]. Importantly, DNA methylation and histone modifications communicate and cooperate as exemplified by recruitment of histone deacetylases to methylated CpG islands and targeting of *de novo* DNA methyltransferases to sites with pre-existing histone methylation (e.g., H3K9 and H3K36 methylation) [567,568]. Accordingly, altered landscape of histone marks has also been reported to contribute to the deregulation of genes encoding transcription factors. In breast cancer, *FOXC1* expression is controlled by a super-enhancer characterised by enrichment of H3K27ac. Deletion of the super-enhancer resulted in decreased expression of *FOXC1* and reduced spheroid growth [569]. In colorectal cancer, *SOX9* becomes transcriptionally activated upon loss of repressive H3K27me3 mark [5]. Additionally, genetic and epigenetic changes may disrupt the higher-order chromatin organisation which leads to transcriptional dysregulation as reviewed in [570]. To emphasise, the DNA and chromatin modifications are deposited and removed by epigenetic modifiers that are recruited to specific genomic loci by transcription factors, therefore transcription factors cooperate with coregulators to drive the epigenetic reprogramming and orchestrate transcriptional activity [570,571]. Nevertheless, the interplay between transcription factors and epigenetic control is complex and reciprocal (e.g., altered chromatin accessibility can have an impact on the set of genes regulated by a transcription factor while not all epigenetic changes are accompanied by a change in transcriptional activity) as discussed in [572–574]. Taken together, the epigenetic profile modulates the expression and determines the target genes of stemness-associated transcription factors.

Third, the stability and translation of a transcription factor mRNA is controlled by epitranscriptomic pathways and RNA interference networks. Epitranscriptomics is the study of biochemical RNA modifications [575]. Accumulating evidence indicates that changes in the epitranscriptome contribute to tumour initiation and progression [576, 577]. The most studied RNA modification is the methylation of adenosine at position 6 to give *N*⁶-methyladenosine (m⁶A) which affects mRNA stability, splicing and translation [253]. In endometrial cancer, *SOX2* mRNA is upregulated as a result of HIF- and ALKBH5-dependent demethylation [2]. Similarly, ALKBH5-mediated demethylation increases the mRNA and protein levels of *NANOG*, and promotes stem-like phenotype in breast cancer [14]. In acute myeloid leukaemia, m⁶A promotes the translation of c-MYC mRNA [8]. The role of other RNA modifications in the deregulation of stemness-associated transcription factors is not completely understood. In addition to the chemical modifications, mRNA stability and translation is regulated by microRNAs (miRNAs), a class of small non-coding RNAs that mediate post-transcriptional gene silencing. The expression of miRNAs and their target genes is dysregulated in human malignancies where the miRNAs targeting tumour suppressors are upregulated, whereas the miRNAs targeting oncogenes are downregulated [578]. In pancreatic cancer tissues, the miR-1181, which targets *SOX2*, is significantly downregulated and there is a negative correlation between the levels of miR-1181 and *SOX2* [4]. In gastric cancer, *GLI2* becomes upregulated due to silencing of miR-144-3p [35]. Importantly, like the crosstalk between epigenetic control and transcription factor expression, the regulation of transcription factor levels by miRNA network is a two-way street – miRNAs target transcription factor mRNAs and genes encoding miRNAs are targets for transcription factors.

Fourth, the protein abundance, subcellular localisation and activity

Table 2

A summary of the transcription factors and their role in stemness.

Factor	Role	Mechanism	Reference
SOX2	Pro-stemness	Transcriptional activation of <i>CCND1</i> , <i>TERT</i> , <i>YAP1</i> , <i>WWTR1</i> , <i>ABCC2</i> , <i>ABCC3</i> , <i>ABCC6</i> , miR-486-5p, miR-10b-5p, <i>SNAI1</i> , <i>SNAI2</i> , <i>TWIST</i> and <i>ZEB1</i>	[59,60,69,81,82, 91,92,96,97,100]
SOX4	Pro-stemness	Activation of autophagy Upregulation of β -catenin, c-MYC and cyclin D1	[92] [61]
SOX8	Pro-stemness	Transcriptional activation of <i>HDAC1</i> , <i>SNAI1</i> , <i>SNAI2</i> , <i>TWIST</i> and <i>ZEB1</i>	[78,98,99]
SOX17	Anti-stemness	Transcriptional activation of <i>FZD7</i>	[85]
MYC	Pro-stemness	Downregulation of <i>CTNNB</i> , <i>CCND1</i> and <i>MYC</i>	[64]
	Pro-stemness	Epigenetic reprogramming or dedifferentiation	[107,108]
	Pro-stemness	Upregulation of <i>SNAI1</i> , <i>ZEB1</i> /2, <i>TERT</i> and HIF-2 α	[111,113]
	Anti-stemness	Metabolic regulation	[115,116]
KLF2	Anti-stemness	Modulation of retinoic acid signalling	[157]
KLF4	Pro-stemness	Transcriptional activation of <i>ALDH1</i> , <i>ITGB4</i> , <i>OCT4</i> , <i>SOX2</i> , <i>NANOG</i> , <i>CD44</i> and <i>CD133</i>	[124,129,130]
	Pro-stemness	Activation of p38 MAPK pathway	[131]
	Anti-stemness	Downregulation of caveolin-1, <i>CD44</i> , <i>MSI2</i> , <i>OCT4</i> , <i>NANOG</i> , <i>CD133</i> , β -catenin and vimentin	[137–140]
KLF7	Pro-stemness	Activation of EMT	[10]
	Pro-stemness	Upregulation of <i>IFIT1</i> /3, <i>DLG3</i> , <i>CD44</i> , <i>SNAI1</i> , <i>ZEB2</i> , vimentin and <i>MMP2</i>	[149,156]
KLF9	Anti-stemness	Downregulation of <i>ITGA6</i> , <i>BMI1</i> , <i>NESTIN</i> , <i>OLIG2</i> and <i>SOX2</i>	[159]
OCT4	Pro-stemness	Transcriptional activation of <i>FOS</i> , <i>JUN</i> , <i>WNT2B</i> , <i>FZD4</i> /8, <i>LEF1</i> , <i>HNRNPM</i> , <i>SNAI1</i> , <i>SLUG</i> and <i>CXCL13</i>	[170,173–175]
NANOG	Pro-stemness	Upregulation of <i>CDK2</i> , <i>CDK6</i> , <i>CCND1</i> , <i>IL-6</i> , <i>TERT</i> , <i>ZEB1</i> , <i>SNAI1</i> /2, <i>MMP2</i> /9, <i>CXCR4</i> , <i>ABCG2</i> , <i>CD44</i> , <i>OCT4</i> and <i>MYC</i>	[191–193,195, 199,202,204,205]
	Pro-stemness	Induction of quiescence	[196]
HIF	Pro-stemness	Upregulation of <i>SOX2</i> , <i>KLF4</i> , <i>SNAI1</i> , <i>SNAI2</i> , <i>ADAM12</i> and <i>ALKBH5</i>	[221,236,238, 240–244,2]
	Pro-stemness	Regulation of cell cycle	[221,246,251]
HIF-1 α	Pro-stemness	Upregulation of <i>CD73</i> , <i>A2BR</i> , <i>DUSP9</i> and <i>COX2</i>	[229,233,234]
	Pro-stemness	Transcriptional activation of miR-1275, <i>SHH</i> , <i>SMO</i> , <i>GLI1</i> , <i>GLI2</i> and <i>TERT</i>	[237,238,254]
HIF-2 α	Pro-stemness	Transcriptional activation of <i>SOD2</i>	[223]
SNAI1	Pro-stemness	Upregulation of <i>NANOG</i> and <i>NEFL</i>	[266,267,271]
	Pro-stemness	Downregulation of <i>let-7</i> and <i>CCN3</i>	[269–271]
	Anti-stemness	Downregulation of <i>TGFB1</i> , <i>ASCL2</i> , <i>TCF7L2</i> , <i>MYB</i> and <i>WNTLRINC1</i>	[274,275]
SNAI2	Pro-stemness	Upregulation of <i>OCT4</i> , <i>NANOG</i> , <i>SOX2</i> , <i>ABCC1</i> –6, <i>ABCG2</i> and <i>IGFBP2</i>	[276,277]
	Pro-stemness	Transcriptional repression of <i>SLC13A3</i>	[278]
TWIST1			[279]

(continued on next page)

Table 2 (continued)

Factor	Role	Mechanism	Reference
ZEB1	Pro-stemness	Transcriptional activation of SOX2	[287,298]
	Pro-stemness	Metabolic plasticity	
	Pro-stemness	Transcriptional repression of NGN3	
	Pro-stemness	Upregulation of OCT4 and NANOG	
SMAD	Pro-stemness	Partial EMT	[305]
SMAD2	Pro-stemness	Regulation of cell cycle and EMT	[308]
SMAD3	Pro-stemness	Transcriptional activation of c-JUN, FOXA2 and ZEB1	[300]
SMAD4	Anti-stemness	Transcriptional repression of ALDH1A1	[310]
β-catenin	Pro-stemness	Upregulation of OCT4, SOX2, c-MYC, NANOG, N-cadherin, vimentin, SNAI2, ABCB1, ABCG2, CCND1, c-JUN, TERT, MMP9, CD133, CD44, MLL1, MACC1, LGR5 and ALDH2	[315–321,331,332,334,339]
YAP/TAZ	Pro-stemness	Upregulation of FOS, FOXO1, FOXO3, SALL1, BCL6 and ERF	[355]
	Pro-stemness	Cellular plasticity	[355]
YAP	Pro-stemness	Upregulation of SOX2/9, NANOG, ALDH1, PDGFB, CD44, CD133, KLF4, OCT4, SALL4, LGR5, MYC, ABCG2, HIF-1α, HK2, ITGB2, TGF-β, TWIST, HDAC2 and serglycin	[346–349,352,353,354,356,357,360,362–364,366]
	Pro-stemness	Cooperation with retinoic acid receptors	[363]
	Pro-stemness	Activation of NF-κB	[361]
	Anti-stemness	Downregulation of Lgr5	[365]
TAZ	Pro-stemness	Upregulation of SOX2, CD44, CD133 and ALDH1A1	[351]
	Pro-stemness	Maintenance of telomere integrity	[367]
FOS	Pro-stemness	Upregulation of NANOG, MYC, SOX2 and NOTCH1	[171,385]
FOSL1	Pro-stemness	Epigenetic reprogramming	[374]
	Pro-stemness	Upregulation of SNAI2, FOSL1, CD44, EPHA2, MCL1, BIRC5, MMP3/8, TAZ and NANOG	[374,378,384]
JUN	Pro-stemness	Epigenetic reprogramming	[375–377]
P53	Anti-stemness	Downregulation of ZEB2, N-cadherin, vimentin, CD44, OCT4, MYC and KLF4	[388]
	Pro-stemness	Upregulation of OCT4, SOX2, NANOG and FOXH1	[392,394]
NOTCH	Pro-stemness	Upregulation of SNAI2	[410,411]
NOTCH1	Pro-stemness	Upregulation of CXCR4, SMAD3 and CD44	[406,410]
	Pro-stemness	Transcriptional activation of SOX9	[408]
STAT3	Pro-stemness	Upregulation of NANOG, SOX2, POU5F1, DOT1L, FOSL1, HLA-FAS1, miR-92a, CPT1B, TWIST and SNAI1	[384,425,427,428,431,433,434]
	Pro-stemness	Regulation of cell cycle	[434,435]
GLI	Pro-stemness	Upregulation of FOXM1, BMI1, SOX2, OCT4 and BCL2	[455–457]
	Pro-stemness	Activation of EMT	[458,459]
GLI1	Pro-stemness	Upregulation of SOX2 and VEGFA	[446–448]
GLI2	Pro-stemness	Transcriptional activation of circ-STAT3	[449]

Table 2 (continued)

Factor	Role	Mechanism	Reference
	Pro-stemness	Downregulation of SOX17	[450]
GLI3	Pro-stemness	Upregulation of SOX2	[448]
ETV7	Pro-stemness	Transcriptional repression of interferon response genes	[466]
ELK-1	Pro-stemness	Transcriptional activation of SOX2	[468]
ESE-1	Anti-stemness	Downregulation of NANOG	[469]
BRACHYURY	Pro-stemness	Upregulation of NODAL, LEFTY, OCT4, PAX6, REX1, NANOG, YAP, SNAI1, SNAI2 and ZEB1	[472,474,477]
HOXA1	Anti-stemness	Downregulation of NANOG	[493]
HOXA13	Pro-stemness	Activation of EMT	[495]
HOXB7	Pro-stemness	Activation of EMT	[494]
	Pro-stemness	Transcriptional activation of Lin28B	[497]
FOXC1	Pro-stemness	Transcriptional activation of β-catenin	[528]
FOXD1	Pro-stemness	Transcriptional activation of SNAI2	
FOXM1	Pro-stemness	Upregulation of OCT4, NANOG and SOX2	[515]
FOXO	Anti-stemness	Regulation of cell cycle	[504]
FOXQ1	Pro-stemness	Sirtuin 1-mediated nuclear translocation of β-catenin	[527]
RUNX1	Pro-stemness	Transcriptional activation of PU.1	[535]
	Anti-stemness	Transcriptional repression of ZEB1	[541]
	Anti-stemness	Formation of inhibitory complex with YAP	[542]
SALL4	Pro-stemness	Upregulation of KHDRBS3, NANOG and OCT4	[548,549]
NF-κB	Pro-stemness	Upregulation of SNAI1, TWIST, BCL-2 and BIRC5	[558]
	Pro-stemness	Maintenance of telomere integrity	[559]
	Pro-stemness	NF-κB/miR-497/SALL4 axis	[560]

A2BR, adenosine receptor 2B; ABCB/C/G, ATP binding cassette subfamily B/C/G; ADAM, a disintegrin and metalloproteinase domain-containing protein; ALDH, aldehyde dehydrogenase; ASCL2, achaete-scute complex homolog 2; BCL-2, B-cell lymphoma 2; BIRC5, baculoviral IAP repeat containing 5 (also known as survivin); BMI1, BMI1 proto-oncogene, polycomb ring finger; CCND1, cyclin D1; CD, cluster of differentiation; CDK, cyclin-dependent kinase; COX2, cyclooxygenase 2; CXCL, CXC chemokine ligand; CXCR, CXC chemokine receptor; DLG3, discs large MAGUK scaffold protein 3; DUSP9, dual specificity phosphatase 9; EPHA2, EPH receptor A2; ERF, ETS2 repressor factor; FZD, frizzled; HDAC, histone deacetylase; HK2, hexokinase 2; IFIT, interferon induced protein with tetratricopeptide repeats; IGFBP, insulin-like growth factor-binding protein; ITG, integrin; KHDRBS3, KH RNA Binding Domain Containing, Signal Transduction Associated 3; let-7, microRNA let-7; LGR5, leucine-rich repeat-containing G-protein coupled receptor 5; MACC1, metastasis-associated in colon cancer 1; MAPK, mitogen-activated protein kinase; MCL1, MCL1 apoptosis regulator, BCL2 family member; miR, microRNA; MLL1, mixed lineage leukemia 1; MMP, matrix metalloproteinase; MSI2, musashi RNA binding protein 2; MYB, transcriptional activator MYB; NGN, neurogenin; OLIG2, oligodendrocyte transcription factor 2; PAX6, paired box 6; PDGFB, platelet derived growth factor subunit B; REX1, reduced expression 1 (also known as ZFP42); SHH, sonic hedgehog; SLC13A3, solute carrier 13 A3; SMO, smoothened; TCF7L2, transcription factor 7-like 2 (also known as TCF4); TERT, telomerase reverse transcriptase; VEGFA, vascular endothelial growth factor A; WNTRLINC1, WNT-regulated long intergenic non-coding RNA 1.

of a transcription factor are controlled by post-translational modifications (e.g., phosphorylation and ubiquitination) and proteolysis. The most common and best characterised post-translational protein modification is phosphorylation which has been shown to regulate the localisation, stability and function of a protein. Phosphorylation and dephosphorylation events play a key role in cellular signal transduction cascades where kinases and phosphatases catalyse the addition and removal of a phosphate group, respectively, to control the activity of substrate proteins [579,580]. Many stemness-associated transcription factors are regulated by signalling pathways and become hyperactivated in cancer due to elevated activation of upstream signalling cascade. For example, the overproduction of Hedgehog ligands leads to aberrant activation of GLI transcription factors while the growth factor-activated PI3K/AKT signalling promotes the activity of NF- κ B transcription factors and inhibits the function of FOXO transcription factor subfamily (reviewed in [439] and [581]). Further, oncogenic KRAS has been shown to activate transcription factor c-JUN via JNK-mediated phosphorylation, and increased phosphorylation of YAP at the position Y357 promotes its nuclear accumulation and thus facilitates its function as a transcriptional regulator [582–584]. In addition to phosphorylation, ubiquitination is an important post-translational modification that marks proteins for degradation via proteasome pathway. The ubiquitin-mediated proteolysis of stemness transcription factors is altered in cancer as reviewed in [585] and [586]. For instance, loss of E3 ubiquitin ligase von Hippel-Lindau (pVHL) stabilises transcription factor HIF-1 α in clear cell carcinomas [587].

In conclusion, transcription factors can become deregulated at DNA, chromatin, RNA or protein level through various intertwined mechanisms. Importantly, transcription factors play a central role in the stemness network consisting of upstream regulators, downstream targets and feedback loops by integrating information from signalling pathways to trigger specific transcriptional response. The expression and activity of transcription factors are tightly regulated via multilevel control which ensures coordinated and nuanced gene expression programs. Intriguingly, transcription factors themselves are part of the regulatory machinery. All of the above leads to three key conclusions. First, due to the presence of feedback loops and cross-regulation between transcription factors, deregulation of one stemness-associated factor has the potential to trigger (at least partial) activation of stemness program. Second, the interconnectedness and crosstalk between individual components (e.g., transcription factors, signalling pathways, epigenetic regulators and metabolites) blur the distinction between cause and consequence in stemness state. Third, mechanisms of deregulation are interlinked. For example, genetic mutation can create a binding site for a transcription factor that has been activated by an upstream signalling. In turn, this activated transcription factor shapes the chromatin landscape and drives expression of another stemness-associated transcription factor.

24.2. Targeting deregulated transcription factors

Since the transcription factors are critical for establishment and maintenance of drug resistant stemness state in cancer, targeting of the deregulated transcription factors holds potential to significantly improve patient outcomes. However, transcription factors have long been considered undruggable due to the structural disorder and the lack of deep pockets for small molecule binding. Notwithstanding, recent years have seen great advances in designing anticancer therapeutics that directly target transcription factors (reviewed in [588–590]).

The approaches to drug transcription factors include targeting protein-protein interactions, disrupting transcription factor-DNA binding, modulating proteasomal degradation of transcription factors, and delivering small interfering RNA (siRNA) or CRISPR-based therapeutics for targeted gene silencing [588,591,592]. The function of transcription factors relies on their ability to complex with other transcription factors, co-regulators and DNA. Targeting these interactions is a promising

therapeutic strategy. For example, PT2385, an inhibitor that interferes with the assembly of HIF-2 α /HIF-1 β heterodimers, achieves disease control in 41% of patients with clear cell renal cell carcinoma [593]. CB-103, a selective inhibitor that prevents the interaction between Notch intracellular domain (NICD) and CSL proteins, has advanced into phase II clinical trials and shown a clear benefit in a patient population with metastatic adenoid cystic carcinoma [594]. Medinger et al. have reported a case of a patient with relapsed T cell acute lymphoblastic leukaemia, who achieved complete remission and became minimal residual disease (MRD)-negative after addition of CB-103 treatment to ongoing therapy [595]. In addition, compounds that bind specific genomic sequences and compete with transcription factors for their consensus binding sites have been developed. For instance, a sequence-selective DNA-binding synthetic molecule PIP-S2 inhibits the interaction of SOX2 with DNA [596]. However, no molecule of this class has progressed to clinical trials for cancer [588]. Alternatively, stemness transcription factors can be targeted by designing chimeric degraders. Proteolysis targeting chimera (PROTAC) is a heterobifunctional molecule consisting of three elements: 1) a ligand that binds to the protein of interest, 2) a ligand that is responsible for the recruitment of E3 ubiquitin ligase, 3) a linker that connects the functional domains. Therefore, PROTAC technology is a novel therapeutic strategy that is used to artificially induce the degradation of the protein of interest [597,598]. A PROTAC that targets STAT3 has been reported to suppress STAT3 transcription network, and achieve complete and long-lasting tumour regression in xenograft mouse models [599]. Lastly, transcription factor expression can be silenced by siRNA or CRISPR-based approaches (reviewed in [591] and [592]). The antisense oligonucleotides targeting MYC (drug name: Oncomyc-NG) or STAT3 (drug name: Danvatirsen) are under investigation in phase II clinical trials [591].

In conclusion, although many challenges remain in targeting the stemness-associated transcription factors, the results from preclinical and clinical studies are highly encouraging [588,589,600].

25. Concluding remarks

The stem-like state in cancer is orchestrated by an intricate network of deregulated transcription factors that underlies the seamless coordination of cellular processes endowing stem-like cells with growth and survival advantage. The network involves a large number of positive feedback loops which together with the partial overlap between transcription factor target genes make the stemness state robust yet plastic (Table 2, Fig. 6D-E). First, the redundancy in target genes demonstrates that the effects of numerous transcriptional regulators eventually converge on a limited number of cellular processes. Second, the unique target genes of each transcription factor allow finetuning of the stem-like state by refining only a particular aspect of the complex stemness phenotype. Third, the network integrates signals from signalling pathways, thus facilitating response and adaptation to microenvironmental cues. Therefore, the stemness in cancer is more nuanced than previously appreciated with possibly more than one type of stem-like cells present in an individual tumour. Finally, understanding the mechanistic links between deregulated transcription factors and stem-like features is essential for designing better therapeutic approaches and demystifying stemness that has proven to be a molecular masterpiece.

Data Availability

No data was used for the research described in the article.

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