

The role of cancer metabolism in defining the success of oncolytic viro-immunotherapy

Arthur Dyer¹, Sally Frost¹, Kerry Fisher¹ and Len Seymour^{1,2}

1 Department of Oncology, University of Oxford, Oxford, UK

2 len.seymour@oncology.ox.ac.uk, Old Road Campus Research Building, Department of Oncology, University of Oxford, OX37DQ

Declaration of Interests

LS and KF holds equity in PsiOxus Therapeutics Ltd. All the other authors declare that they have no conflict of interest.

Funding Information

AD, KF and LS are funded by Cancer Research UK (CRUK, C557/A17720). S.F. is supported by MRC Centre for Doctoral Training grant number MR/N013468/1. A.D. is supported by a Graduate Scholarship from Jesus College, Oxford.

Author Contributions

Arthur Dyer: Conceptualisation, writing - original draft, writing – reviewing & editing **Sally Frost:** Conceptualisation, writing - original draft, writing – reviewing & editing **Kerry Fisher:** Conceptualisation, supervision **Len Seymour:** Conceptualisation, writing – reviewing & editing, Supervision, Project administration.

Abstract

Oncolytic viruses infect, replicate in, and kill cancer cells selectively without harming normal cells. The rapidly expanding clinical development of oncolytic virotherapy is an exciting interdisciplinary field that provides insights into virology, oncology, and immunotherapy. Recent years have seen greater focus on rational design of cancer-selective viruses together with strategies to exploit their immunostimulatory capabilities, ultimately to develop powerful oncolytic cancer vaccines. However, despite great interest in the field, many important experiments are still conducted under optimum conditions *in vitro*, with many nutrients present in excess and with cellular stress kept to a minimum. Whilst this provides a convenient platform for cell culture, it bears little relation to the typical conditions found within a tumour *in vivo*, where cells are often subject to a range of metabolic and environmental stresses. Viral infection and cancer will both lead to production of metabolites that are also not present in media *in vitro*. Understanding how oncolytic viruses interact with cells exposed to more representative metabolic conditions *in vitro* represents an under-explored area of study that could provide valuable insight into the intelligent design of superior oncolytic viruses and help bridge the gap between bench and

bedside. This review summarises the major metabolic pathways altered in cancer cells, during viral infection and highlights possible targets for future studies.

Introduction

Modified metabolism as a hallmark of cancer

It has long been documented that cancer cells have altered cellular programming which results in increased survival, growth and proliferation leading to tumour formation and progression. These key changes were documented in the seminal review “The Hallmarks of Cancer” by Hanahan and Weinberg in 2000 which highlighted the underlying principles that govern the transformation of normal cells to cancer cells [1]. This review highlighted the genetic and epigenetic changes that induce stem-cell-like properties, such as unlimited cell division and blocked differentiation. In 2011 the same authors released an updated list with two new hallmarks; immune evasion and deregulated metabolism, both of which are fundamental to the success of oncolytic virotherapy [2].

One well studied feature of metabolic reprogramming in cancer is the “Warburg Effect” in which tumour cells increase glucose consumption and lactate secretion, even in the presence of oxygen [3]. This provides several key advantages to cancer cells. Notably the rate of metabolism through aerobic glycolysis occurs 10-100 times faster than oxidative phosphorylation such that the amount of ATP synthesised over a given period is comparable or even greater [4]. Against this background, the metabolic plasticity in most cancer cells allows for alternative metabolites (such as glutamine) to be used for biosynthetic purposes and it has been shown that malignant cells require a high metabolic flux to synthesise building blocks such as nucleotides, proteins and lipids. This anabolic phenotype induces a unique microenvironment that includes decreased concentrations of nutrients and oxygen, and increased levels of harmful metabolic waste products such as the production of lactate which inhibits many immune cell functions. All of which contributes towards formation of an “immunologically cold” cancer, which is difficult to treat and is associated with poor prognosis.

Lytic viruses as anticancer agents

Oncolytic virotherapy has recently received renewed interest since promising clinical results for the first agent (Imlygic) which received its product licence in the USA and Europe in 2015 [5,6]. The current upsurge of interest in oncolytic viruses (OVs) reflects the culmination of incremental scientific progress gained over several years that is now being translated into important clinical progress [6–9].

Many wild-type viruses show an intrinsic selectivity for replication within cancer cells. The mechanism underlying this is a topic of intense discussion and is likely to reflect the activated nature of tumour cells, providing a cellular phenotype that is conducive to efficient virus replication. Many publications highlight that there appears to be considerable overlap between the hallmarks of cancer and the hallmarks of virus infection.

As viruses are obligate intracellular pathogens, they need to hijack cellular processes to create a favourable environment for efficient production of infectious progeny. In many ways their demands for macromolecular synthesis appear similar to those of a proliferating or cancer cell. Metabolomic analysis of virally infected cells was first conducted with cells infected with human cytomegalovirus (HCMV) [10]. During the lytic portion of its lifecycle, HCMV was found to induce a range of metabolic changes including an upregulation of the tricarboxylic acid (TCA) cycle, nucleotide biosynthesis, and glycolysis. Such alterations are now known to be critical for replication of many viruses, including Kaposi's sarcoma-associated herpesvirus, herpes simplex virus 1, adenovirus, hepatitis C virus, human immunodeficiency virus and dengue virus [10–15].

As with tumour cells, infection of cells with viruses induces a unique metabolic microenvironment including decreased concentrations of nutrients, such as glucose, glutamine, arginine, tryptophan and decreased oxygen, and an increase in metabolic waste products, such as lactate, glutamate, and reactive oxygen species (ROS).

Oncolytic virotherapy and metabolism

The success of oncolytic virotherapy relies on the ability of the virus to enter and replicate successfully within tumour cells that are exposed to the many stressful environments listed above. However, there are surprisingly few publications investigating how oncolytic viruses cope with unfavourable metabolic conditions. As a notable example, the levels of glucose used *in vitro* typically range from 5-25 mM whereas in clinical cancers the glucose concentrations can be as low as 0.1-0.4 mM, even with an average blood concentration of around 6 mM [16]. Equally, lactate levels intratumourally can rise to levels of 40 mM, which is far higher than normally present in *in vitro* conditions [17].

In nearly all published studies, cancer cells were grown in these non-pathological conditions with nutrients like glucose far in excess of what is to be found even in the blood circulation. The impact of high levels of glucose on these experiments is a key consideration for future studies because it is crucial to test new drugs targeting cancer cells under physiologically relevant conditions. One such example is that of metformin (a drug currently being tested for a wide range of cancer treatments) which has been shown to be more effective in enhancing the sensitivity of cancer cells to chemotherapy only when cells are grown in reduced glucose conditions [18]. In 2016, Pusapati *et al* showed that weaning cancer cell lines off of glucose makes them significantly more susceptible to co-treatment with mTORc inhibitors and anti-glycolysis drugs [19].

Glucose and glutamine metabolism

The recent advances in the field of metabolomics have led to a much deeper understanding of metabolism of non-cancerous cells, tumour metabolism and how pathogenic agents hijack cellular metabolism in order to meet their own energetic and biosynthetic demands. Both cancerous and virus-infected cells show a phenotype of increased uptake and catabolism of nutrients. Infectious viruses of many types have been shown to reprogram

various aspects of host cell central carbon metabolism including increased glycolysis, elevated pentose phosphate pathway and increased lipid and amino acid synthesis. Whilst several viruses upregulate consumption of key nutrients like glucose and/or glutamine and converge on a similar metabolic pathway, the precise metabolic changes induced by specific viruses is often context-dependent and can vary even within families of viruses or cell types that are infected. Whilst we do not aim to encompass the entire field of viral metabolism in this brief review, we have focused on the metabolic challenges associated with some of the viruses that present promising opportunities for oncolytic virotherapy.

DNA Viruses

Herpesviruses

In the 1950s Eagle and Habel showed that replication of poliovirus (a positive single stranded RNA virus) in HeLa cells was abrogated in culture media composed solely of a balanced salt solution. Upon addition of glucose to the salt solution viral proliferation and viral loads were restored to normal levels, suggesting that glycolysis was essential to allow poliovirus replication [20]. These findings were subsequently confirmed in primary monkey kidney cells where an increase in lactate in the media during the first two hours of infection was shown [21,22]. As early as 1962 it was shown that replication of Herpes Simplex Virus 1 (HSV-1, an enveloped double-stranded DNA virus) was also dependent on glucose in HeLa cells [23].

Members of the Herpesviridae family of viruses are, to date, the most studied viruses in terms of metabolism. For HSV-1, in 1962 it was shown that in the absence of glucose, viral entry into host cells was not affected but that no infectious progeny was produced [23]. It was shown that HSV-1 virion production did not decrease in the presence of 2-Deoxy-d-glucose (2DG, a competitive inhibitor of glucose metabolism) but that production of infectious particles was severely impaired, possibly due to glycosylation changes in the viral glycoproteins on the envelope of the virus [24]. Similar results were also shown using another alphaherpesvirus, pseudorabies [25].

When the host cell response to infection with HSV1 was analysed via metabolomics it was found that with HSV-1, only around 1% of glycolytic TCA cycle intermediates were derived from glycolysis and that TCA intermediates were instead provided largely by a flow of glutamine via α -ketoglutarate [12]. A possible explanation for this dramatic change in metabolism is that glucose uptake may have been increased to feed the pentose phosphate pathway to support increased nucleotide synthesis, leaving a demand for other metabolites to be produced from an alternative source such as glutamine [27,28].

These results highlight a few important lessons. Firstly, the metabolic demands of one virus in a particular cell type is not the requirement for all viruses, even if they are closely related

and secondly, despite these differences, the metabolic requirements of these viruses can be defined to quite specific accuracy.

Vaccinia Virus

Vaccinia virus, a cytoplasm-based enveloped double stranded DNA poxvirus, provides an interesting case point in the current literature regarding metabolomics during viral infection. Studies on vaccinia virus infected human foreskin fibroblasts showed no increase of glycolytic intermediates, and this led Fontaine *et al* to hypothesise that glycolysis is not necessary for vaccinia virus replication [29]. This was confirmed by the removal of glucose from the media of vaccinia virus infected cells, resulting in no effect on the levels of replication and production of infectious progeny. This indicated that utilisation of glycolysis for viral replication is evidently not universal [29]. Further studies have shown that vaccinia virus replication is highly dependent on glutamine and that restriction of glutamine or inhibition of glutaminolysis inhibits the vaccinia virus lifecycle [29,30]. It has recently been shown that this dependence on glutamine was due to expression of the viral protein C16 which stabilises hypoxia inducible factor 1 alpha (HIF1 α) [31].

Adenoviruses

Adenoviruses are a diverse family of non-enveloped double stranded DNA viruses. Shortly after they were discovered, it was observed that infection with adenoviruses causes an increase in glycolysis and lactic acid production [32–34]. Many members of the adenovirus family have been shown to induce glycolysis through expression of an early viral gene, E4ORF1, which interacts with Myc causing it to translocate to the nucleus and specifically upregulate metabolic genes involved in glutamine and glucose metabolism [34,35].

Interestingly, in cells that have a low glycolytic rate, knockdown of c-Myc decreases viral titres but not in cells with a high glycolytic rate [35]. The observation that adenoviruses can respond to glycolytic alterations differently in cells that have a high glycolytic rate compared to those with a low glycolytic rate was confirmed in a 2018 paper released from our laboratory which showed that less glycolytic cells tend to be less permissive to viral oncolysis and that restriction of glycolysis through pharmaceutical inhibition or through repeated culture in low glucose conditions lead to an improved viral lifecycle [34].

With respect to adenoviruses and glutamine metabolism, it is evident from the research listed above that glutamine metabolism is vital for viral replication and that inhibition of glutamine metabolism dramatically inhibits viral replication, a fate that adenoviruses also share with HSV-1 and influenza [36]. However boosting glutamine metabolism and, more specifically reductive carboxylation (a metabolic pathway whereby glutamine can be metabolised outside of mitochondria without the requirement for oxygen) can improve viral oncolysis [34,36].

Interestingly, human adenovirus species D type 36 and 31 are prevalent in obese individuals and infection with type 36 human adenovirus has been associated with metabolic changes in animal models [37–39]. These metabolic changes have been linked again to the early viral

protein E4ORF1 and are the subject of many research papers investigating whether there is a causal link between the expression of this protein and obesity with some claiming that adenovirus 36 infection promotes weight gain both *in vitro* and *in vivo* [37–41]. further research conducted in murine and avian models showed that infection with Ad36 resulted in a statistically significant weight gain [38] however, infection with Ad36 also resulted in an improved glycaemic control [37,42,43]. These alterations were later found to be attributable to the E4ORF1 gene and its interactions with phosphatidyl inositol 3-kinase (PI3K) and with MYC [44–46] leading many research groups to suggest the potential of E4ORF1 as a template for developing antidiabetic therapies because of its ability to strongly control glycolysis to favour the pentose phosphate pathway. The metabolic reprogramming of host cells by adenoviruses is reviewed in greater detail in a 2019 paper by Prusinkiewicz and Mymryk highlighting the considerable overlaps between the metabolic profiles of adenovirus infections and the Warburg effect [47].

RNA Viruses

HIV

One virus which highlights some of the difficulties in studying viral metabolomics is the Human Immunodeficiency Virus 1 (HIV-1). HIV-1 is an enveloped single stranded positive sense RNA virus that is classified as a non-transforming lentivirus. A metabolomic study of HIV-1 infected macrophages and CD4⁺ T Cells showed an interesting result. In macrophages, which generally maintain a long-term (latent) infection, infection with HIV-1 resulted in a decreased uptake of glucose and a decrease in glycolytic metabolite accumulation [14]. In CD4⁺ T Cells, which generally support an acute lytic infection, the presence of HIV-1 resulted in the opposite metabolic profile with infected cells showing increased glycolysis [14,48]. These findings highlight the importance of the host cell model used to study metabolomics and echo findings in other models suggesting that latency and acute infection can show markedly different metabolic profiles in closely related viruses [49,50].

Influenza

Many other viruses either directly induce glycolysis during infection or cause host cells to upregulate glycolysis at various stages during infection such as several strains of influenza A, a single stranded, segmented negative strand RNA virus, which induces glycolysis at a relatively late stage of infection (coinciding with the onset of apoptosis) [51]. In 1959, the glycolysis inhibitor 2-DG was shown to inhibit viral replication *in vitro* in line with the findings that influenza increases glycolysis [52]. This finding, however, was contrasted in 2016 by Wang *et al* who showed that treatment with 2-DG in an *in vivo* influenza model decreased overall survival by increasing viral replication [53]. The authors suggested that this decrease in host survival was likely due to the fact that 2-DG treatment will result in deregulated unfolded protein response (UPR). These contrasting differences between a cellular level of control by the virus and an organism level of homeostasis and metabolism highlight the divergent metabolic needs of the different cell types within an organism.

Rhinovirus

Rhinovirus, a small RNA virus which belongs to the *picornaviridae* family and is the causative agent of the common cold. It has been shown that, as with most viruses, a response of host cells to infection with rhinoviruses is an increased glucose uptake and that the virus is dependent on both glutamine and glucose for optimal replication [54]. It is interesting to note that, unlike most DNA viruses mentioned above, this increase in glucose uptake occurs as early as 1.5 hours post infection meaning that this response to infection occurs before viral proteins can be translated meaning this is a host cell response to viral infection and not caused by expression of a viral protein.

Rhabdoviruses

Rhabdoviridae is a family of enveloped group V viruses (single stranded negative RNA genomes) of which we will focus on Vesicular Stomatitis Virus (VSV) because this virus is the most common member of the family used in oncolytic virotherapy. VSV is able to replicate in a wide number of cancer cells both in normoxic and hypoxic conditions making it an attractive platform for oncolytic virotherapy [55]. Previous studies have shown that VSV replication is enhanced in cancer cells when they are grown in malignant ascites fluid as these conditions increased the glycolysis of certain cell lines meaning these cells increased lactate production and elevated the use of glutamine as the predominant carbon source [56]. Indeed, inhibition of glycolysis or glutaminolysis has been shown to decrease oncolytic VSV replication however this can be rescued by addition of oxidative TCA intermediates when glycolysis is inhibited or through addition of α -ketoglutarate when glutaminolysis is inhibited [56].

Paramyxoviruses

Viruses in the family of *Paramyxoviridae* are negative-sense, single stranded RNA viruses that include measles, mumps and Newcastle disease virus (NDV). Little is currently known about how NDV alters metabolism in cells, however, in 2014, Deng *et al* suggested that NDV might reduce the activity of host glycolysis by decreasing the glycolytic enzyme phosphoglycerate kinase (PGK) [57]. Despite the paucity of information on the metabolic alterations of cells infected with NDV, the virus seems highly selective for cancer cells and is displaying promise as an oncolytic agent [58]. As with NDV, relatively little is known about how measles viruses affect the metabolism of their host cells but a paper in 2014 reported that infection of cells with the oncolytic measles virus Edmonston strain (MV-Edm) upregulates glycolysis under aerobic conditions (the Warburg effect) in glioblastoma cells, which was evidenced by increased glucose uptake, lactate production, and LDHA expression [59].

Viral metabolic demands conclusion

Overall, although the metabolic response of host cells to viral infections have overlapping commonalities, the metabolic demands for each virus are varied and even closely related viruses can have different requirements. However, each virus has clear metabolic demands and metabolic consequences for the microenvironment, summarised in table 1.

Despite this seemingly widespread increase in glucose uptake during viral infection there are some marked differences between both the causes of this increase and the benefits to the viruses. Some viruses actively increase glucose uptake whereas in other cases, the host cells increase glucose uptake in response to the presence of the virus. It is also worth noting that despite infection with viruses causing an upregulation of glucose uptake, inhibition of glycolysis or removal or restriction of glucose during infection can have a wide variety of effects on the virus lifecycle, as will be discussed later.

Glutamine metabolism in general and the reliance of virally infected cells upon glutamine is less studied than glucose in the field of virology and even less so in oncolytic virotherapy. In general, many cancer cells are considered to be “glutamine addicted” meaning they use glutamine as a source of metabolites, in part compensating for the lack of oxidative glucose metabolism. Glutamine is often catabolised by reductive carboxylation and can be used by cells for a range of purposes including producing ATP, amino acids, nucleotides, fatty acids, mTOR activation, the hexosamine biosynthesis pathway, as well as protecting cells against ROS [60–62]. Because of these factors it is undoubtedly an essential nutrient for many OV and further research needs to be done to understand whether altering glutamine metabolism can improve the outcome of oncolytic virotherapy.

Restriction of metabolites during cancer therapy

The reduction of nutrients or the removal of metabolites from media, or the use of metabolic inhibitors provides important insights for the field of virology in general and holds particular importance for the field of oncolytic virotherapy. In humans, a blood glucose concentration of 6 mM is considered average and a blood glucose level of about 7.1 mM is considered hyperglycaemic. In many cases, the glucose concentrations observed in bodily compartments can be much lower than the levels observed in the blood.

There is an increasing amount of scientific literature in both pre-clinical and clinical settings showing that fasting can sensitise malignant cells to cancer treatments whilst at the same time protecting non-malignant cells in a phenomenon that is referred to as the differential stress response (DSR) [63]. The principal reasoning that is postulated to explain the DSR is that during tumourigenesis various mutations are gained including the ability to proliferate in unfavourable conditions without the presence of external growth factor signalling and that these cells therefore become prone to stressful conditions such as chemotherapy when deprived of nutrients as they are not able to respond to the protective signals generated by fasting [2]. This response is in contrast to non-malignant cells which, during calorie restrictions or fasting have been shown to respond favourably (figure 2). Preclinical studies

have shown that intermittent fasting can extend lifespans, delay type II diabetes and cardiovascular and neurodegenerative diseases as well as reducing cancer rates [64–68].

There are various clinical trials and pre-clinical projects investigating the effects of calorie restriction (reducing intake to 75% fewer calories) and short-term starvation (no food intake) on cancer treatments due to the apparent weakness of cancer cells to restricting nutrients and the benefits to normal tissues. Preliminary reports have found that fasting for between two to five days can protect patients from the side effects of chemotherapy without causing chronic weight loss [68]. This is in contrast to calorie restriction which requires weeks or months to be effective and causes more modest changes in glucose levels in both humans and rodents [69].

With respect to preclinical investigations into the effects of the DSR in cancer treatments, Raffaghello *et al* published in 2008 that starvation in mice allowed researchers to increase the tolerable dose of chemotherapy up to three times higher than the maximal dose approved in humans [70]. Lee *et al* published in 2012 that 15 out of 17 mammalian cancer cell lines tested are sensitised to various chemotherapeutics by fasting 24 hours prior to and 24 hours post-chemotherapy [71]. This paper also showed that two cycles of fasting in mice was as effective as two cycles of chemotherapy alone in reducing tumour burden and that the combination of starvation plus chemotherapy was the most effective regimen [71].

To date there have been a small number of clinical trials that have researched the effect of combined fasting and chemotherapy in patients mainly focussing on the reduction in side effects of chemotherapy. Safdie *et al* in 2009 reported a case study with 10 patients showing reduced chemotherapeutic side effects when fasting 48-140 h prior and 5-56 h post treatment [71]. Other similar reports are reviewed by De Groot *et al* [69]. There are many larger clinical trials currently ongoing to determine the possible benefits of fasting with respect to treatment efficacy, adverse events, quality of life and changes in metabolic, hormone and inflammatory response (NCT00936364, NCT01802346, NCT02710721, NCT03162289, NCT03340935, NCT03595540, NCT03709147, NCT03700437, NCT01175837).

Restriction of metabolites during oncolytic virus therapy

There have been only a limited number of studies investigating the effects of restricting metabolites or fasting on oncolytic virotherapy, although they have all shown highly promising results. The first such paper written by Esaki *et al* showed that transient fasting improved the replication of oncolytic herpes virus in glioblastoma models with striking results shown both *in vitro* and *in vivo* [72]. In 2019, Scheubeck *et al* reported that low glucose, low serum starvation has the potential to enhance measles virus-mediated oncolysis efficacy in HT-29 cells, in terms of a synergistic effect [73].

We reported in 2019 that restriction of glucose or inhibition of glycolysis improved viral DNA replication, viral protein synthesis and the rate of cell lysis after infection with an oncolytic adenovirus in glucose-dependent cell lines and that removal of glucose does not inhibit infection in highly permissive cancer cells [34]. We also reported that cells that were previously poorly permissive to oncolytic adenovirus became highly permissive after repeated culture in low glucose media or in the presence of glycolytic inhibitors.

Despite little being known about how paramyxoviruses alter metabolism, there have been some highly promising results studying the benefits of restricting metabolism during infection with OV in the family *Paramyxoviridae*. In 2014, it was reported that that attenuated oncolytic measles virus Edmonston strain (MV-Edm) caused glioblastoma cells to shift to high-rate aerobic glycolysis; this adaptation was blocked by dichloroacetate (DCA), an inhibitor of glycolysis, leading to profound cell death of cancer cells but not of normal cells. DCA enhanced viral replication by mitigating mitochondrial antiviral signalling protein-mediated innate immune responses [59]. Two groups have investigated the inhibition of glycolysis during oncolytic treatment with NDV. They independently found that 2-DG and NDV synergise to kill breast cancer cells by inhibition of the glycolysis pathway through GAPDH downregulation [74], and that the pyruvate dehydrogenase kinase inhibitor DCA not only promotes NDV replication in a similar manner but, excitingly appeared to improve the immune response to NDV oncolysis by removing immunosuppressive microenvironmental factors such as kynurenine due to IDO1 expression and lactate [75].

These papers highlight the possibilities of combining OVs with metabolism-altering treatments and are only possible through rigorous understanding of the metabolic demands of each virus platform. What is true for one virus will not be true for all and so any use of glycolytic inhibitors or intermittent fasting needs to be evaluated for each viral platform. Further uses of understanding metabolic interactions and OVs will be discussed below.

Future Directions

As discussed above, reduction of metabolites to more clinically relevant levels can have a marked effect upon how cancer cells respond to stressors such as OVs. Whilst modelling precisely the environment encountered *in vivo* can seem daunting or even impossible, there are methods that can be adopted to, at least attempt to bridge the gap between current culture conditions and those encountered in a tumour. The reduction of metabolites such as glucose and/or glutamine to much lower levels is a simple prospect that that be readily incorporated into most *in vitro*, cancer models. Many groups have found promising results through repeated culture of cells in low metabolite conditions because, at first, some cells will not respond well to a decline in these previously abundant metabolites and likely require time to adjust their metabolism to be able to cope with the more stressful conditions [34,76]. Nevertheless, a number of studies have shown that previously highly glycolytic cell lines when cultured for even a small amount of time in low glucose conditions completely alter their metabolic profiles [77–79].

Gaining an understanding of the metabolic requirements of cells infected with your OV of choice is of key importance for a number of reasons. Firstly, it could allow for a more targeted therapy of cancers. Many techniques such as positron emission tomography (PET) imaging and single-cell analysis have advanced such that we can gain insights into the metabolic landscape of tumours before treatment and this along with advancements in genotyping could allow for screening of patients with a tumour that will support OV treatment. Likewise,

understanding the metabolic requirements of cells infected with any given OV can highlight limiting metabolites that are required for effective oncolysis allowing a supplementation of these through dietary means, through co-treatment with metabolic drugs designed to increase specific metabolic pathways, or through the design of armed OVs that express enzymes or drugs designed to target the metabolic environment.

In considering cancer as a whole, it would be remiss to not consider the effects of altered metabolism following oncolytic virus infection on the immune status of tumours. Little work is currently conducted into the effects of OVs upon the immune system specifically focussed on the effects of altered metabolism and how that changes the microenvironment. A large number of OVs will increase both nutrient competition and metabolic waste accumulation. Whilst it is true that viral infection has been shown to increase the presence of immunostimulatory markers such as DAMPs and PAMPs and can cause immunogenic cell death of host cells, it remains unclear if this is able to outcompete the immunosuppressive environment that viruses may encourage due to exaggeration of the Warburg effect or an increase in suppressive metabolites already found in the tumour microenvironment. Understanding what level of effect these factors exert over immune cell function will only improve the design of OVs especially those armed to encourage immune cell stimulation.

Some promising examples of harnessing metabolism to improve oncolytic virotherapy have been published in the past two years. One such example is the finding that inhibiting glycolysis during infection with oncolytic Newcastle disease virus appears to improve the immune response by removing immunosuppressive factors such as IDO1 and lactate [75]. An exciting advancement in the field of immunometabolism was published recently highlighting the possibility of using OVs as a vector to alter metabolism in favour of viral replication and improved immune cell stimulatory phenotypes. Rivadeneira *et al* in 2019 reported that the immune response to OVs was incomplete due to metabolic insufficiencies induced by the tumour microenvironment and this was exacerbated by the presence of the virus. The authors then showed that the adipokine, leptin, was able to metabolically reprogramme T cells and that engineering OVs to produce leptin within the tumour induced complete responses in tumour-bearing mice and supported memory development in the tumour infiltrate [80].

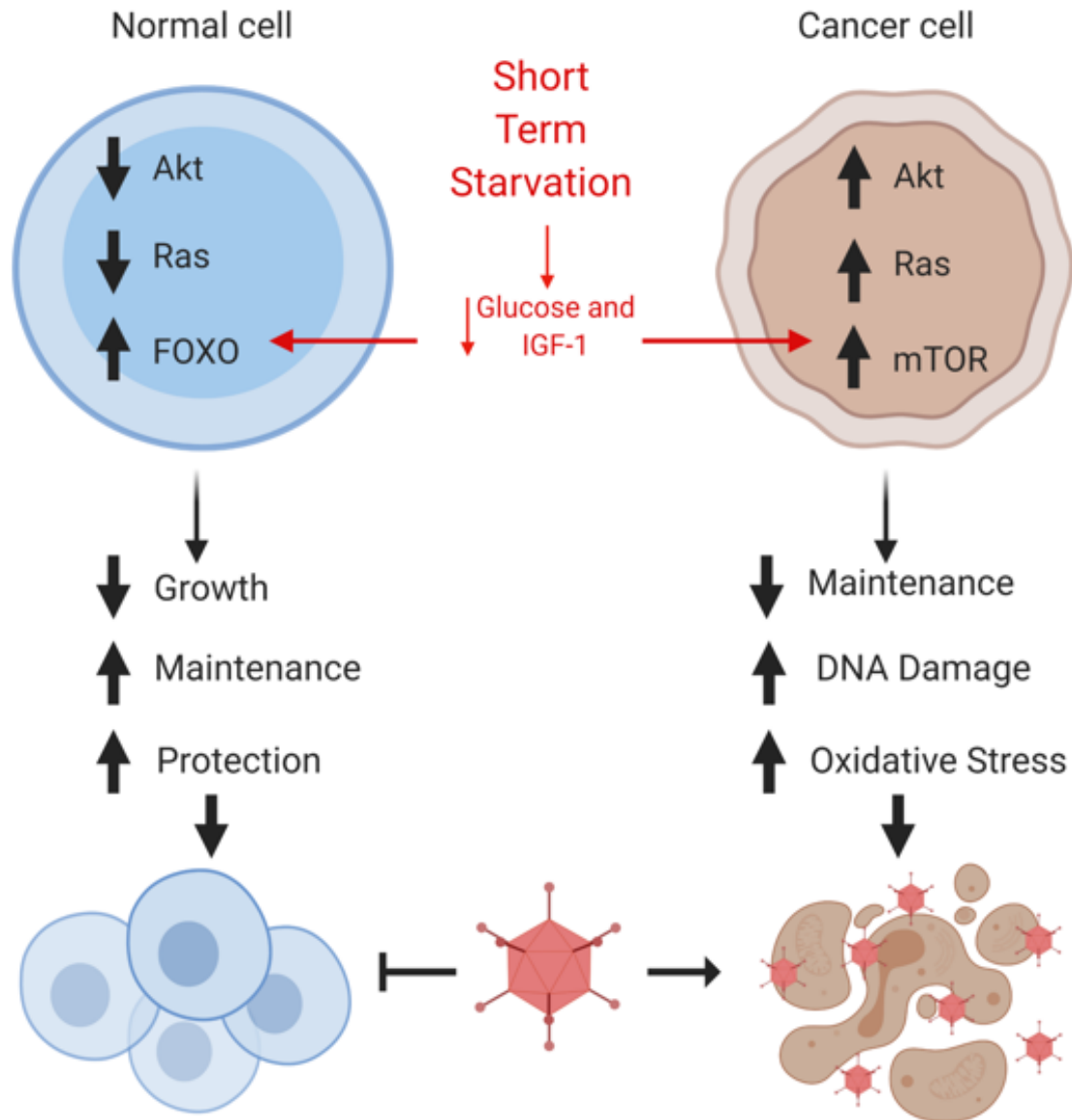
Conclusions

Cancer cells, immune cells and the surrounding milieu that make up the tumour microenvironment all play a critical role in the success or failure of any anti-cancer drug and this is particularly true of oncolytic viruses which rely on the behaviour of cancer cells for their success. Recently there have been a number of papers showing that cancer cell lines behave differently depending upon the metabolic environment in which they are cultured [18,19]. Several studies have also indicated that either through starvation or through

restriction of certain nutrients, cancer cells can become more vulnerable to lysis with OV or can improve the ability of the virus to replicate within these cells with dramatic effects being seen in situations or cell lines where OVs previously struggled to perform. Certainly, even a relatively simple step of reducing glucose concentrations *in vitro* can dramatically alter the response of cancer cells to stressors and, we believe, should be considered a vital step in bridging the gap between the bench and bedside.

Despite the fact that metabolic requirements differ based on which virus is used, each OV requires a specific, yet definable, intracellular metabolic environment and they provide an obvious and, as yet, underexplored platform with which to produce these environments through the expression of metabolic reprogramming agents. Furthermore, the immune response to OV infection may be dampened through immune cell competition for nutrients with virally infected cells or through the increase in metabolic waste from virally infected cells. OVs which can alter the tumour microenvironment to favour both virus infection and to create a more immune-stimulating environment represents an exciting step forward in the field of viro-immunotherapy with some promising results beginning to emerge to support this theory [34,59,72–75].

Differential Stress Response



Differential Stress Response in cancer treatment. Simplified schematic representation of the differential stress response. Short term starvation decreases serum levels of glucose and insulin-like growth factor (IGF-1) which affect downstream growth regulators including mammalian target of rapamycin (mTOR), Akt, Ras and Forkhead Box O proteins (FOXO). In cancer cells, these regulators are activated in response to the short-term starvation, increasing oxidative stress and decreasing cell maintenance, leading to DNA damage and making them more susceptible to lysis by oncolytic viruses, whereas the opposite occurs in non-cancerous cells.

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