

MYB is activated in CYLD defective Cylindromas and contributes to tumour proliferation *in vitro*

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Running title: MYB is frequently overexpressed in familial dermal cylindromas

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Abstract

Skin cylindromas are rare tumours usually characterised by a benign nature, but that can occasionally give rise to malignant cylindrocarcinomas. These tumours could occur sporadically or be symptomatic of Brooke-Spiegler syndrome (BSS), a rare inheritable condition characterized by the mutation of the gene CYLD. Rajan et al. have shown that the oncogenic transcription factor MYB and some of its target genes are often upregulated in cylindromas derived from BSS patients when compared with their normal skin counterparts. Conversely, the fusion Protein MYB-NFIB, commonly observed in sporadic cylindromas, is not present in the CYLD defective tumours cohort. MYB RNA interference in cells derived from BSS patients leads to a reduction of cell proliferation, suggesting that the activation of MYB plays a pivotal role in the development of these tumours. The authors suggest a link between defective CYLD and MYB overexpression, and identify MYB activation as a common feature in cylindromas and a potential target for therapeutic intervention.

Cutaneous cylindromas are benign tumours deriving from skin appendages that might occur as single tumours (sporadic cases) or as multiple cylindromas (familial cases) most commonly in the head and neck. The familial cylindromatosis, also defined as Brooke-Spiegler syndrome, is associated with the mutation of the tumour suppressor gene CYLD (Bignell et al., 2000). Malignant transformation of cylindromas are rare, but are often characterized by high grade and metastatic behaviour, which drastically reduces the survival (Caputo & Rongioletti, 2015). The treatment for these neoplasms is limited to broad margin excision or high-dose radiotherapy for unresectable tumours (Caputo & Rongioletti, 2015). A deeper understanding of the pathophysiology of Cylindromas could be beneficial in finding new means of therapeutic intervention for patients affected by these tumours.

It has been suggested that the loss of heterozygosity in the CYLD locus accounts for the majority of both familiar and sporadic cylindromas, but the molecular pathways involved in

the growth of these tumours are still poorly characterized (Bignell et al., 2000; Leonard et al., 2001). Histological and morphological similarities with Adenoid Cystic Carcinomas (ACC) led to hypothesize that these two tumours could share certain molecular features. Indeed, it was previously shown that sporadic cylindromas and the majority of ACCs, often bare the MYB-NFIB translocation and/or stain positive for MYB (Fehr et al., 2011). The abnormal activation of the transcription factor MYB was firstly shown to transform haemopoietic cells in vitro and in vivo and later was found to be involved in the development of several other malignancies (Gonda, 1998). In ACC, it has been suggested that the recurrent fusions of MYB with the transcription factor NFIB might disrupt MYB's negative regulation leading to MYB overexpression; an alternative proposed mechanism consists in the transposition of super-enhancers deriving from the fusion partner gene to the MYB locus that could lead to its overactivation (Drier et al., 2016; Persson et al., 2009). It is intriguing to speculate that given the histological similarities between ACC and cylindromas, the upregulation of MYB could be a common mechanism underlying both neoplasms.

In their paper, Rajan et al. investigated the role of MYB in CYLD defective cylindromas and spiroadenomas (Rajan et al., 2016); their finding suggest that MYB activation could be an important event in the physiopathology of these tumours. Since they previously observed an high incidence of MYB-NFIB fusion transcripts in sporadic cylindromas (Fehr et al., 2011), they analysed a cohort of samples deriving from CYLD defective familial tumours, but they did not detect any MYB-NFIB fusion transcripts, nor copy number gain involving the MYB locus. However, immunohistochemical analysis revealed that MYB is expressed in the majority of the CYLD defective tumours (69 %) and the expression is significantly higher when compared to adjacent normal skin. To assess the functional significance of MYB activation in tumour growth, the authors transfected siRNAs targeting MYB into primary cultures and carried out proliferation assays. The reduction in MYB expression resulted in a significant decrease in cell proliferation. These findings identify a novel role of MYB in familial cylindromas and suggest the existence of a link between CYLD and MYB. CYLD is a

deubiquitylating enzyme that regulates different target proteins by removing polyubiquitin chains from specific substrates. CYLD loss has been shown to increase cell survival or cell proliferation in different cell types, supporting its role of tumour suppressor gene (Massoumi, 2010). Although at present there are no direct evidences of the interaction between CYLD and MYB pathways in cancer, the authors suggest that a possible link can be found in the activation of Nf-kB. Indeed, CYLD downregulation was previously shown to increase Nf-kB signalling, and MYB could be trans-activated upon Nf-KB activation (Brummelkamp, Nijman, Dirac, & Bernards, 2003; Suhasini & Pilz, 1999). However, the authors reported that they did not observe any effect on MYB expression following NF-kB inhibition in cylindromas cells, suggesting that another mechanisms could be involved. These results suggest that loss of heterozygosity in the CYLD locus might lead to MYB activation, and that when CYLD is intact, other factors such as MYB-NFIB fusions in sporadic tumours could be pivotal in the development of cylindromas. The authors also conducted a gene expression study on previously published microarray datasets, finding that MYB, and two MYB target genes, BCL2 and BIRC3, were upregulated in cylindromas when compared to normal skin. Moreover, knock down of MYB with siRNA reduces the expression of both BCL2 and BIRC3. These finding suggest that MYB is likely to drive proliferation in cylindroma cells trough the inhibition of apoptosis.

Although this work provides new important insights into the physiopathology of familial cylindromas, few questions still remain unaddressed. For instance, it is still unclear whether there is a link between CYLD and MYB activation. It would be interesting to investigate whether MYB or MYB target genes expression varies upon the downregulation/overexpression of CYLD, and to investigate potential convergent pathways.

Moreover, several sporadic cases of cylindroma were also reported to have loss of heterozygosity in correspondence of the CYLD locus (Biggs, Chapman, Lakhani, Burn, & Stratton, 1996). Since the authors previously reported that MYB-NFIB fusions are frequently present in sporadic cylindromas, it would be interesting to assess whether these

chromosomal rearrangements overlap with defective CYLD; this should clarify whether MYB-NFIB fusions, or MYB activation alone, are sufficient to drive the growth of cylindromas in the absence of CYLD mutations.

Taken together, Rajan et al. findings extend the knowledge on a poorly characterised tumour and open new scenarios of possible therapeutic intervention, particularly for malignant cylindromas. MYB targeted therapies are very promising and are recently attracting interest for its potential use in multiple malignancies. For example, in a recent paper from Dai et al. it was shown that the multi-kinase inhibitor rigosertib, which underwent multiple clinical trials, is able to induce selective cytotoxicity in diffuse large B-cell lymphoma models by suppression of TRAF6 and MYB activity (Dai, Hung, Chen, Lai, & Chang, 2016). TRAF6 is an adaptor protein that was shown to participate in promoting tumour development with different mechanisms (Han, Zhang, Qiu, & Yi, 2016). Interestingly, TRAF6 was previously shown to be a CYLD target protein (Sun, 2010); in the light of the findings of Rajan et al., it would be interesting to test rigosertib or other MYB inhibitors in preclinical models of cylindroma. A deeper characterization of the potential cross-talk between CYLD and MYB pathways could also provide more insights into the development of these tumours and uncover new meanings of therapeutic intervention, particularly for aggressive, metastatic cylindromas.

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