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INVITED COMMENTARY

Cutaneous cylindroma: it's all about MYB#

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#Invited commentary for Rajan N, Andersson MK, Sinclair N, *et al.* Overexpression of *MYB* drives proliferation of *CYLD*-defective cylindroma cells. *J Pathol* 2016; **239:** 197–205. AQ2

Abstract

Cutaneous cylindroma is a rare benign tumour that occasionally turns into malignant cylindrocarcinoma. The cancer can be sporadic or emerge in the context of Brooke–Spiegler syndrome (BSS), an inheritable condition characterized by mutation of the gene *CYLD*, encoding a tumour suppressor protein that controls the activity of the transcription factor NF-kB. Sporadic cylindromas present histological features shared with adenoid cystic carcinoma (ACC), a head and neck cancer originating from salivary or other exocrine glands. Like ACCs, sporadic cylindromas express, although at lower frequency, the aberrant fusion transcript MYB–NFIB. In a paper recently published in the *Journal of Pathology*, the research teams led by Neil Rajan and Goran Stenman demonstrate that CYLD-defective cyclindromas in BSS patients are negative for the MYB–NFIB fusion. Only the wild-type MYB oncoprotein is activated in the majority of these tumours. RNA interference studies in cells derived from BSS patients indicate that ablating MYB expression results in a striking reduction of cylindroma cell proliferation, suggesting that MYB plays a pivotal role in the biology of this cancer. The take-home message of the study is that activation of MYB, in its wild-type form or fusion derivatives, is a common feature of spontaneous and hereditary cylindromas, constituting a potentially actionable therapeutic target.

Keywords: MYB; cylindroma; Brooke–Spiegler syndrome; CYLD; adenoid cystic carcinoma

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Familial cylindromatosis, also defined as Brooke–Spiegler syndrome (BSS), is associated with mutation of the tumour suppressor gene *CYLD* [1]. Malignant transformation of cylindromas is rare, but often results in the development of high-grade metastatic tumours that drastically reduce patient survival. The treatment for these neoplasms is limited to broad margin excision or high-dose radiotherapy for unresectable tumours. A deeper understanding of the pathophysiology of cylindromas is essential if new useful therapeutic targets are to be identified.

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 deregulated in these tumours are still poorly characterized [1,2]. Histological and morphological similarities with adenoid cystic carcinomas (ACCs) led to the hypothesis that the two cancer types might harbour common molecular alterations. Indeed, a fraction of sporadic cylindromas and approximately half of ACCs express the *MYB–NFIB* fusion gene [3]. c-MYB (hereafter indicated by MYB) is a transcription factor encoded by a gene belonging to a small family that also includes *MYBL2* (encoding B-MYB) and *MYBL1* (encoding A-MYB). They share a DNA binding domain that recognizes the consensus sequence C/TAACNG, frequently observed in the enhancers of genes associated with cell cycle progression, regulation of cell survival and lineage specification [4]. It is likely that spatio-temporal distribution, more than structural differences, explains the requirement of the different MYBs in organism and tissue development. There is a growing body of evidence suggesting that MYB proteins play an important role in human cancer, with different family members mutated or activated in leukaemia, neuroblastoma, brain, colon, liver and breast cancers [5–8]. The majority of ACCs display rearrangements of the *MYB* locus, with recurrent fusions of MYB with the transcription factor NFIB [9]. More recently, it has been shown that *MYB–NFIB* fusion-negative, but *MYB* locus rearranged, ACCs display activation of *MYB* caused by the translocation of super-enhancers near the gene [10]. Thus, activation of *MYB* might explain the similar histological and morphological features of ACCs and cylindromas.

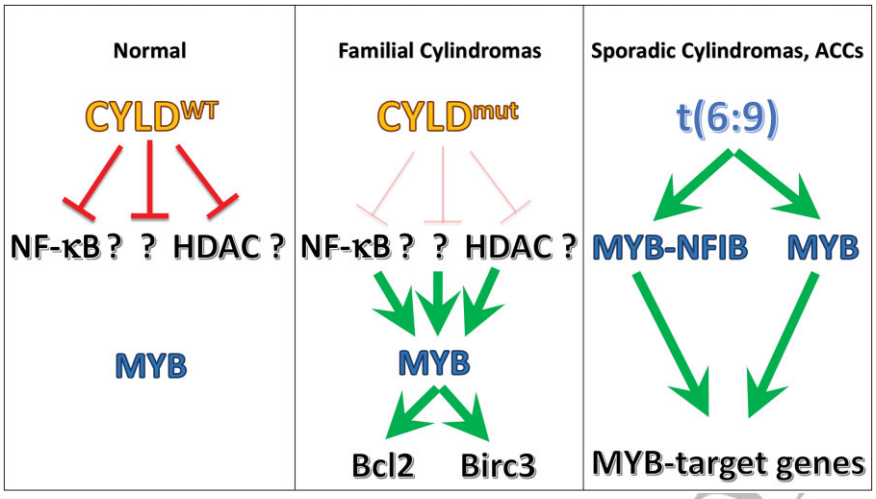

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Figure 1. Different modalities of MYB activation in familial and sporadic cylindromas: (left panel) in normal epidermal cells the tumour suppressor gene *CYLD* inhibits NF-kB, HDACs and possibly additional molecules and pathways (indicated by the question marks); (centre panel) mutations of *CYLD* in familial cylindromas disrupt the block on NF-kB, HDACs and/or other molecular pathways, leading to the activation of *MYB* (green arrows) and the anti-apoptotic proteins Bcl2 and Birc3; (right panel) in sporadic cylindromas and ACCs the t(6:9) or other translocations involving the *MYB* locus activate expression of the MYB oncoprotein via the formation of fusion genes or epigenetic rearrangements

In a study recently published in the *Journal of Pathology*, Rajan *et al* [11] investigated the role of *MYB* in CYLD-defective cylindromas and spyroadenomas. The same group previously observed a relatively high incidence of *MYB–NFIB* fusion transcripts in sporadic cylindromas [3]. Surprisingly, when the researchers analysed a cohort of samples deriving from CYLD-defective familial tumours, they did not detect *MYB–NFIB* fusion transcripts or rearrangements of the *MYB* locus. However, immunohistochemical analysis revealed strong nuclear expression of MYB in the majority of BSS tumours. Importantly, they verified that the expression of the oncoprotein is significantly higher in cancer than in normal skin. To verify the functional significance of MYB activation in cylindromas, the research team implemented RNA interference experiments in which they depleted the expression of *MYB* in primary tumour cultures derived from patients. Reduced *MYB* expression caused a significant decrease in tumour cell proliferation. These findings confirm that overexpression of MYB is a key feature of familial cylindromas and link the mutation of the tumour suppressor gene *CYLD* with MYB activation. Heterozygosity of the *CYLD* locus has been observed in a fraction of sporadic cases of cylindroma [12]. Since *MYB–NFIB* fusions are also observed in these tumours, it would be interesting to assess whether these chromosomal rearrangements are mutually exclusive with *CYLD* alterations. This would corroborate the hypothesis that increased expression of MYB, either as MYB–NFIB fusion or wild-type protein, is the causative event in these tumours.

CYLD is a de-ubiquitylating enzyme that regulates protein stability by removing poly-ubiquitin chains from substrates. CYLD loss has been shown to promote survival or proliferation of different cell types, supporting the hypothesis that it may act as a tumour suppressor. Prior to the study by Rajan *et al* [11], there was no evidence of a link between MYB and CYLD pathways in cancer cells. The authors of the study suggest that a possible explanation for the activation of *MYB* in *CYLD* mutant cells may rest in the loss of control of NF-kB activity. Indeed, *CYLD* inactivation causes increased NF-kB signalling and it was previously reported that the *MYB* promoter contains NF-kB binding sites, transactivated by NF-kB [13,14]. Perplexingly, however, Rajan *et al* [11] did not observe perturbation of *MYB* expression after drugging NF-kB in cylindroma cells, suggesting that another circuitry linking *CYLD* and *MYB* must be operating in cutaneous tumours. It is tempting to speculate that *CYLD* could alter chromatin dynamics in the *MYB* locus, since recent studies have revealed that *CYLD* negatively controls the activity of histone deacetylases HDAC6 and HDAC7 in mammalian cells [15,16]. Intriguingly, the pan-HDAC inhibitor Givinostat has been shown to strongly down-regulate MYB expression in leukaemic cells, indicating that histone acetylation changes might be crucially linked to *MYB* activation in cancer. This hypothesis is corroborated by a study demonstrating epigenetic activation of the *MYB* locus in MYB–NFIB-negative, but translocation-positive, ACCs [10]. Taken together, these studies strongly indicate that the pathogenic cause of cylindromas and ACCs is the activation of *MYB*.

Of course, there are still important questions awaiting an answer: is MYB necessary and sufficient for the transformation of cutaneous and glandular cells? What are the critical MYB target genes? To start answering the latter question, Rajan *et al* [11] conducted gene expression analyses on previously published microarray datasets. Among others, they detected two MYB target genes

involved in the control of apoptosis, *BCL2* and *BIRC3*, which were significantly up-regulated in cylindromas compared to normal skin. Satisfyingly, ablation of MYB reduced the expression of *BCL2* and *BIRC3* in cylindroma cells, suggesting that MYB also precipitates cutaneous tumourigenesis through inhibition of apoptosis. Whether or not MYB is the key driver of cyclindroma, or other head and neck cancers, will only be established by developing appropriate transgenic models or by implementing DNA-editing strategies that reproduce the genomic rearrangements leading to *MYB* activation.

These findings of Rajan *et al* [11] give hope to patients affected by malignant cylindroma. Small-molecule inhibitors of MYB are being developed, some of which show promise in preclinical experiments. For example, the multi-kinase inhibitor Rigosertib induces selective killing of diffuse large B cell lymphoma by suppressing TRAF6 and MYB [17]. Interestingly, TRAF6 is an adaptor protein involved in tumour development and was previously shown to be a *CYLD* target protein [18]. It will be important to assess whether Rigosertib kills or reduces the proliferation of cylindroma cells in preclinical experiments.

Author contributions

Both authors were involved in preparing the manuscript.

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