The potential for divergent biology from one tumor-derived GIC/GSC population to another has profound therapeutic implications. For example, it was demonstrated that BMP2/4 induces terminal differentiation in GICs/GSCs, leading to the suggestion that BMP2/4 and/or their agonist might represent a therapeutic strategy for gliomas (Piccirillo et al., 2006). When GICs/GSCs from a larger set of GBMs were evaluated, however, it was found that about 20% of GBMs harbor GICs/GSCs with aberrant methylation of the BMP receptor 1b (*BMPR1b*) promoter converting BMP2/4 from a differentiation agent to a mitogenic agent (Lee et al., 2008). Thus, not only would a BMP2/4 agonist fail as a therapeutic strategy in these tumors, it might actually promote GIC/GSC expansion and tumor growth. In fact, these same BMPR1b-methylated GICs/GSCs are LIF unresponsive secondary to the failure of LIF to induce phosphorylation of STAT3 in these cells. These GICs/GSCs (and their parental tumors) are therefore likely to be refractory to TGF-β and thus are unlikely to benefit from therapeutic inhibition of TGF-β and/or the JAK-STAT pathway. Indeed, in the context of a very early embryonic NSC phenotype, STAT3 inhibition is required for cells to exit symmetric cell division and terminally differentiate. Thus, any therapeutic strategy aimed at blocking the JAK-STAT pathway in GICs/GSCs derived from, or with a phenotype similar to, a very early NSC could actually cause expansion of the GIC/GSC pool.

Targeting stem cell pathways may ultimately prove to be an effective therapeutic strategy against malignant gliomas; however, such pathways may have dramatically divergent roles in GIC/GSC populations from different GBMs. Thus, regardless of whether GICs/GSCs come from a true NSC or from the dedifferentiation of a mature somatic cell (e.g., astrocyte), understanding the divergent signaling mechanisms and phenotypes of developmentally and anatomically specified normal NSCs will be vital for understanding the unique biology of GIC/GSC populations from different malignant tumors (Figure 1). Once those pathways have been elucidated and validated as potentially useful therapeutic targets, we will ultimately need to identify easily assessed surrogate molecular markers of stem cell pathway activation within each specific tumor (e.g., BMPR1b, TGF-β/LIF expression) if we want to use such information to guide therapeutic decision-making for individual patients. The promise of patient-specific stem cell pathway therapeutic targeting will therefore be best realized through an ever greater collaborative effort between neuroscientists, developmental biologists, cancer researchers, and clinical scientists.

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Fingering Modulators of Retinoic Acid Signaling Identifies New Prognostic Marker for Neuroblastoma

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Properly designed genome-wide screening strategies can provide new insights into biological processes and/or biomarkers for malignant diseases. In this issue of *Cancer Cell*, Huang et al. demonstrate that the Krüppel zinc-finger protein ZNF423 is critical for retinoic acid signaling and is likely a favorable prognostic marker for neuroblastoma.

Vitamin A is essential for development and homeostasis in all vertebrate organisms. All-*trans* retinoic acid (all-*trans* RA) is a key biologically active derivative of vitamin A that can be isomerized into 9-*cis* RA and 13-*cis* RA forms (compounds collectively known as retinoids). The biological actions of both natural and synthetic retinoids are mediated via

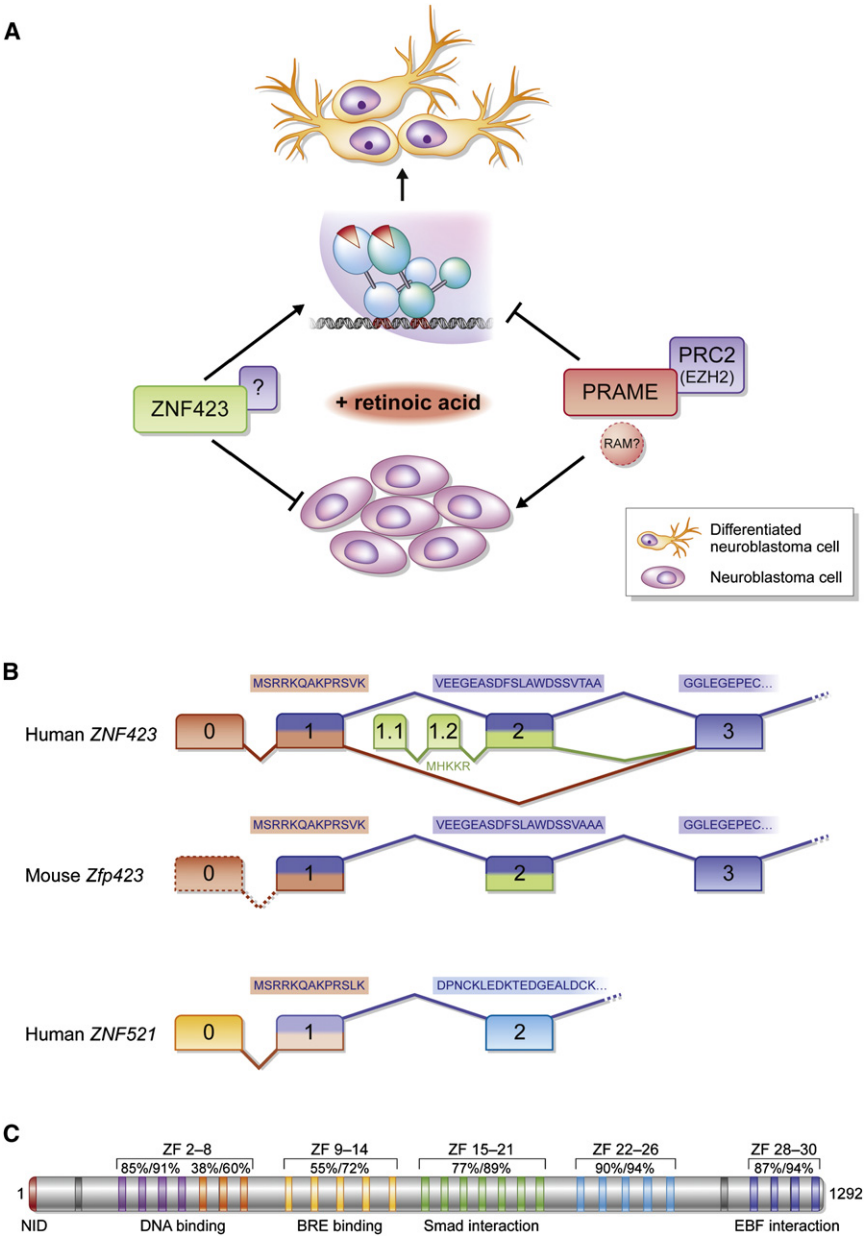

Figure 1. Modulators of Retinoic Acid Signaling

(A) Schematic representation of the role of ZNF423 in retinoic acid signaling and neuroblastoma differentiation. Retinoic acid acts via the RXR/RAR heterodimeric complex (center) to induce neuroblastoma cells (bottom) to undergo differentiation (top). ZNF423 can interact with RXR/RAR and stimulate their transcriptional activities as well as the differentiation of neuroblastoma cells. PRAME represses RAR-mediated gene transcription through recruitment of the PRC2-associated EZH2 histone methyltransferase. Whether ZNF423 competes with PRAME for RAR binding or recruits positively acting epigenetic factors in this cell-type context remains to be established.

(B) Schematic representation of the predicted 5′ gene structure of *ZNF423*, *Zfp423*, and *ZNF521*. Bioinformatic analysis (courtesy of Kevin Petrie) suggests that *ZNF423* may be alternatively spliced as well as being transcribed from two distinct putative promoters. In analogy with Zfp423, ZNF423 shares an N-terminal NuRD interaction domain (NID), with the sequence MSRRKQAKPRSVK (related or identical sequences in different proteins are highlighted with the same colors), with several other zinc-finger transcription factors (Bond et al., 2008). ZNF423 also shares a high degree of overall homology with ZNF521: although the N-terminal regions of these two proteins are less homologous, the NID is conserved. Mouse *Zfp423* exon 0 is drawn in dashed outline to indicate that although mRNA corresponding to this exon has not been detected, a sequence homologous to human *ZNF423* exon 0 is present in the *Zfp423* locus. Accession numbers of mRNA sequences used for the above analysis were taken from the NCBI Entrez Nucleotide database: ZNF423, NM_015069, BX403293, DB298660; Zfp423, NM_033327; ZNF521, NM_015461.

(C) ZNF423 and ZNF521 are structurally highly related, comprising an N-terminal NID (red bar) and discrete clusters made up of 30 and 29 Krüppel-like C_2H_2 zinc fingers (ZF, indicated by vertical bars), respectively. A schematic representation of the full-length NID-containing isoform of ZNF423 is shown, with the ZF clusters highlighted by different colors (ZF 2–8, which bind DNA, can be viewed as one or two clusters). Functional properties of other ZF modules are indicated below the diagram as described previously (see Hata et al., 2000). Identities/similarities between the corresponding clusters of ZNF423 and ZNF521 are indicated as percentage values above the diagram. The two vertical dark gray bars represent an N-terminal atypical ZF not present in ZNF521 and a poorly conserved ZF that does not form part of the C-terminal cluster.

retinoic acid receptors (RARs). As members of the nuclear receptor family, RARs act as ligand-regulated transcription factors directly or indirectly to modulate the expression of complex gene networks. RARs function as heterodimers formed from one of three RARs and one of three retinoid X, or rexinoid, receptors (RXRs). In the absence of agonists, RAR/RXR heterodimers bind to some target genes as corepressor complexes and silence their transcription by histone modification. The binding of an agonist replaces corepressors with coactivators within the RAR/RXR complex, thus leading to initiation of transcription through establishment of positively acting chromatin modifications and interactions with the basic transcriptional machinery. Besides this relatively well-characterized general mechanism of coregulator exchange in modulating the activities of nuclear receptors, the function of RARs is regulated by additional cell/promoter context-specific factors. These RA signaling modulators (RAMs) exert diverse but highly efficient effects on RA signaling. For example, *RAM* has been shown to be silenced by RA in the myelomonocytic lineage, and its continuous expression can confer resistance to RA-induced differentiation and apoptosis (Yin et al., 2006). Interestingly, *RAM* is unlikely to encode a protein. PRAME is another negatively acting RAM that binds RAR and blocks its agonist-induced activation, possibly by recruitment of the polycomb PRC2 complex (Epping et al., 2005) (Figure 1A).

In a study in this issue, by performing a large-scale RNA interference-based genetic screen, René Bernards and colleagues identify the zinc-finger protein ZNF423 as a positively acting RAM (Huang et al., 2009). Furthermore, they show that ZNF423 can interact with all RAR subtypes as well as RXR and is required, at least in some cells, for effective transcriptional activation induced by RA. In contrast to PRAME, the binding of ZNF423 to RARs and RXRs does not require ligand. However, the mechanistic basis explaining why ZNF423 is critical for RAR/RXR signaling remains unclear (Figure 1A). Future studies will have to address whether ZNF423 can bind together with RAR/RXR to particular composite DNA elements, has the properties of a coactivator, facilitates corepressor release, and/or recruits additional factors. In this context, it is interesting to note that (1) ZNF423 acts as a BMP4-induced coregulator of the Smad1/4 complex (Ku et al., 2006) and (2) the DNA binding and protein interaction domains of ZNF423 have been mapped (Tsai and Reed, 1998). That the zinc fingers involved in these different signaling paradigms can be separated indicates a functional specification of these structural elements that merits further scrutiny (see Figures 1B and 1C for illustration).

Ever since Wolbach and Howe demonstrated in 1925 that vitamin A deficiency in rats leads to the development of squamous metaplasia, retinoids and more recently also rexinoids have been at the forefront of research into the development of novel differentiation and apoptosis-based cancer therapies (for a recent review, see Altucci et al., 2007). With the exception of acute promyelocytic leukemia, where all-*trans* RA has been an overwhelming therapeutic success, the promise of these drugs in cancer therapy remains to be fulfilled, particularly in solid tumors. Retinoids and rexinoids have, however, yielded encouraging results for acute myeloid leukemia (AML) when used in combination with other signaling drugs (Altucci et al., 2005). Furthermore, the addition of 13-*cis* RA as maintenance therapy has significantly improved the outcome of patients with a high-risk form of neuroblastoma (Matthay et al., 2009), and combination with histone deacetylase inhibitors may further increase therapeutic efficacy (Hahn et al., 2008).

Collectively, the results of these and other studies strongly suggest that the poor response of some tumors to retinoid/rexinoid-based therapies may be due at least in part to an imbalance between positively and negatively acting RAMs in cancer cells. Interestingly, the Huang et al. study shows that high expression of ZNF423 correlates with good outcome of neuroblastoma patients, possibly reflecting enhanced levels of RA signaling. Consistent with this hypothesis, the expression of PRAME, which inhibits RA signaling, has been associated with poor outcome of neuroblastoma patients. It will be interesting to investigate whether the accuracy of prognosis in this disease can be improved by using both ZNF423 and PRAME as markers. It also remains to be determined whether expression of ZNF423 can predict a better response to RA treatment, possibly even in relapsed and therapy-resistant disease. One important finding of the Huang et al. study is that the introduction of ZNF423 into RA-resistant neuroblastoma cell lines restores their differentiation response to RA, indicating that finding ways to elevate ZNF423 levels may be therapeutically useful. The authors point out that DNA methylation inhibitors have no effect on ZNF423 expression, but one cannot exclude that other modifications, perhaps even PRAME/EZH2-mediated trimethylation of histone H3 lysine 27, silence this gene. In this respect, DZNep (3-deazaneplanocin A), a compound that targets the EZH2-containing polycomb complex for degradation, may prove to be useful in restoring some ZNF423 expression and/or activity.

A potential role for ZNF423 in other cancers and their sensitivity to RA also remains to be explored. A highly conserved member of this family, ZNF521, is expressed (potentially aberrantly) in a majority of AML (Bond et al., 2008). An interesting caveat here is that ZNF521 binds the repressive nucleosome remodeling and deacetylase (NuRD) complex and may in fact constitute one of the growing number of factors that inhibit RA response in AML. Although the ZNF423 cDNA used in the Huang et al. study did not contain this NuRD interaction domain (NID), an analysis of genomic and expressed sequence databases indicates that human ZNF423 can be expressed as several isoforms, one of which possesses the NID (Figure 1C). Interestingly, it appears that the mouse gene can only express the NID-containing isoform, complicating the interpretation of the F9 and embryonic stem cell data in the current study. However, it is possible that despite possessing a NuRD-binding motif, ZNF423 does not bind NuRD or have any repressive potential in the context of the RAR complex. With respect to neuroblastoma, it is worth noting that expression sequence tags for ZNF423 transcripts that encode the NID were isolated from neuroblastoma cells and cerebellum. Therefore, it may be important to establish prevalence and relative ratio of the isoforms possessing or lacking the NID, as well as activity on all-*trans* RA signaling in neuroblastoma cells, as this may give further insight into the pathogenesis of this disease, providing clinicians with a better prognostic tool in addition to opening new avenues for translational research.

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