PERSPECTIVE

Pleiotrophin fights Brd2 for neuronal differentiation

Bromodomain containing 2 (Brd2) protein belongs to the Bromodomains and Extra Terminal domain (BET) family of chromatin adaptors characterized by the presence of two N-terminal tandem bromodomains and an exclusive C-terminal extra terminal domain (ET) (Belkina and Denis, 2012; Shi and Vakoc, 2014). Bromodomains are involved in recognizing acetylated histone tails and other acetylated proteins while the ET domain has been implicated in protein-protein interaction. Besides the ability of bromodomains to recognize acetylated chromatin, appropriated dimerization through a coiled coil structure called motif B has been demonstrated essential for proper BET function (Garcia-Gutierrez et al., 2012). A peculiarity of some BET members consists in its ability to remain attached to the mitotic chromosomes, while most of proteins, including other bromodomain proteins, detach from chromatin, what has led to suggest an epigenetic function for BET proteins. Motif B is also required for this ability (Garcia-Gutierrez et al., 2012). Four members conform the mammalian BET family: Brd2, Brd3, Brd4 and Brdt (**Figure 1**). Excepting Brdt, whose expression is restricted to the male germ line, rest of members are widely expressed during development and in the adult. Several approaches have demonstrated the involvement of Brd2 and Brd4 in cell cycle progression (reviewed in Belkina and Denis, 2012; Shi and Vakoc, 2014). These include analysis of knock out mice, knockdown experiments in cell cultures and analysis of protein binding to promoters and enhancers of cell cycle-associated genes. This feature tightly links some BET proteins to a variety of cancers. Indeed, tumor progression in these cancers has been successfully arrested by using synthetic drugs able to displace BET proteins from the chromatin (Shi and Vakoc, 2014). These drugs, usually mimicking acetylated lysines, work by blocking BET bromodomains.

Interestingly Brd2, besides being expressed in proliferating progenitors of the nervous system, is also detected in differentiating neurons (Crowley et al., 2004). Paradoxically, overexpressed Brd2 impairs neuronal differentiation, probably due to stimulation of cell cycle progression, since elevated levels of both cyclin A2 and D1 are maintained under differentiation conditions (Garcia-Gutierrez et al., 2012). Thus, these results raise the question of how the cell deals to exit the cell cycle for neuronal differentiation in the presence of a cell cycle-stimulating protein such as Brd2. The answer relies on pleiotrophin.

Pleiotrophin (*Ptn*) is a growth factor abundantly expressed in the nervous system during development. *Ptn* together with its homologue midkine (*Mdk*) conforms a family of secreted heparin-binding proteins. These molecules have been proved to act as mitogens, to promote cell survival, neurite outgrowth and nerve cell migration, and to display transforming and angiogenic activities in different cell types (Muramatsu, 2011). Thus, *Ptn* and *Mdk* have been associated with neural development and neurodegenerative diseases as well as with cancer. In the classical model, secreted *Ptn* works by interacting with cell surface receptors. Accordingly, *Ptn* is well detected extracellularly and in the cytoplasm. Four transmembrane types of receptors have been indicated as responsible for *Ptn*/*Mdk* signaling: receptor protein tyrosine phosphatase (RPTP)β/ζ, anaplastic lymphoma kinase (ALK), N-syndecan and low-density lipoprotein receptor-related protein-5 (Papadimitriou et al., 2009). However, there is evidence of *Ptn* internalization *via* interaction with low affinity receptors in the cell surface. The nucleolar-associated protein nucleolin has been suggested as such a receptor (reviewed in Papadimitriou et al., 2009). Nucleolin is also able to target *Ptn* to the nucleus, suggesting that *Ptn* displays nuclear functions. This leads to ask whether nuclear functions of *Ptn* are cell autonomous or not, since it is reasonable to assume that *Ptn*, once expressed, may either be secreted or directly enter the nucleus. Whether physiological relevance relies on intracellular *Ptn* targeted to the nucleus or on secreted *Ptn*, internalized in neighbor cells, remains to be investigated. In addition, despite evidence of internalization, intracellular mechanisms of action of *Ptn* have not been elucidated before. In this regard, our lab has shown that *Ptn* is able to interact with Brd2 in the nucleus (Garcia-Gutierrez et al., 2014).

In P19 cells, detection of *Ptn*, both in the cytoplasm and in the nucleus, follows induction of neuronal differentiation (Garcia-Gutierrez et al., 2014). Therefore, *Ptn* and Brd2 interact under neuronal differentiation conditions. Notably, *Ptn* and *Mdk*, as well as *Brd2*, are highly expressed in the central and peripheral nervous systems of the mouse embryo at the mid-gestation stage (ca. E11.5). Significant expression levels are detected in the brain, spinal cord and dorsal root ganglia (Papadimitriou et al., 2009; Shang et al., 2009; Muramatsu, 2011). In the developing neural tube proliferation occurs in the ventricular zone (close to the lumen), and progenitors exiting the cell cycle migrate to the mantle layer (at the pial surface) to differentiate into neurons. The post-mitotic cells in the way of migration conform the subventricular mantle layer (SML). Interestingly, coincident and enhanced expression of *Ptn* or *Mdk* with *Brd2* is observed at the SML in spinal cord sections (Juarez-Vicente and Garcia-Dominguez, unpublished observations). Although *Brd2* is able to interact with both Ptn and *Mdk*, it is the interaction with Ptn what acquires biological significance in P19 cells, as Ptn is the family member detected in these cells when differentiate (Brunet-de Carvalho et al., 2003). In this model, Ptn downregulation significantly impairs neuronal differentiation, while Ptn overexpression favors it. Conversely, *Brd2* impairs neuronal differentiation, being Ptn able to neutralize *Brd2* effect. Interestingly, interfering with *Brd2* by other means leads to the same effects as provoked by Ptn overexpression, indicating that Ptn works by antagonizing *Brd2*. It is important to remark that, at least in mammals, Ptn does not appear to display neurogenic activity, but to enhance neuronal differentiation under induction conditions. Double *Ptn*/*Mdk* knockout mice display severe phenotypes in contrast to mild phenotypes displayed by single mutants,

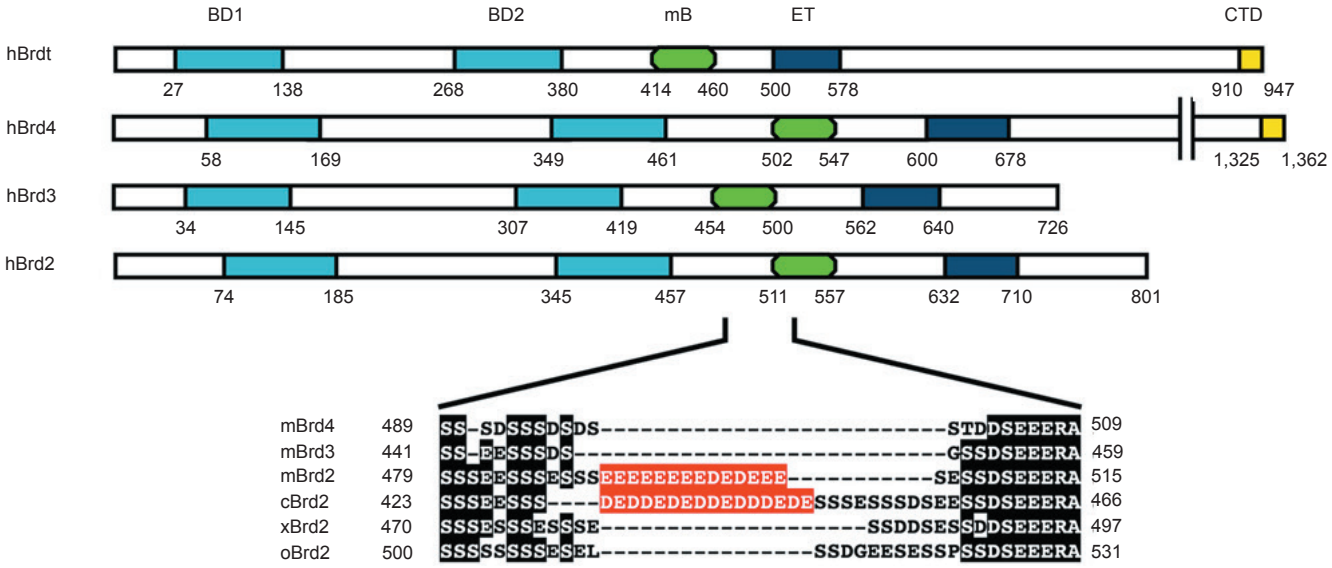

Figure 1 BET family of proteins.
The four human BET members are depicted and relevant domains (BD1, bromodomain 1; BD2, bromodomain 2; mB, motif B; ET, extra terminal domain; CTD, C-terminal domain) indicated. Numbers under each protein correspond to amino acid positions. Sequence of acidic stretch mediating Ptn interaction is also shown (red boxes). Alignment of amino acid sequences encompassing this region for mouse (m) Brd4, Brd3 and Brd2, chicken (c) Brd2, Xenopus (x) Brd2 and Medaka (o) Brd2 has been displayed. Numbers flanking the sequences correspond to amino acid positions. Residues conserved in 4 out of the 6 sequences are boxed in black. Accessions, respectively: NP_065254, NP_001107045, NP_034368, NM_001025845, NP_001128282, BAD93258. Aligments were performed with ClustalX 2.0.11. BET: Bromodomains and extra terminal domain; Brd2: bromodomain containing 2.

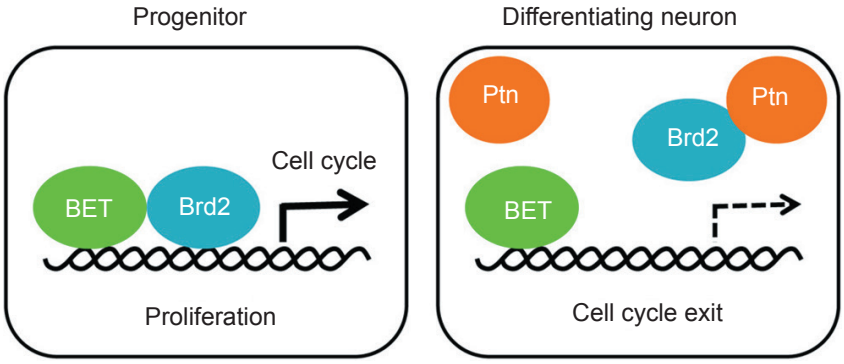

Figure 2 Ptn modulates the balance between proliferation and differentiation during neurogenesis.
Schematic representation of Ptn effects on neural progenitors following induction of neurogenesis. In progenitors, Brd2 and other BET members assure cell cycle progression. In differentiating neurons, Ptn is expressed and antagonizes Brd2, unbalancing the cell towards cell cycle exit and differentiation.

what points to functional redundancy between Ptn and *Mdk* (reviewed in Muramatsu, 2011). However, in agreement with a role of Ptn in favoring neuronal differentiation, single *Ptn* knockout mice show defects in timing of neuronal differentiation in the cerebral cortex. As a consequence of this delayed differentiation, an increased number of progenitors accumulates in the cortex (Hienola et al., 2004). Thus, Ptn emerges as a *Brd2* modulator, which helps to unbalance the cell towards neuronal differentiation once this process is triggered (**Figure 2**). Antagonism between Ptn and *Brd2* is not restricted to differentiating neurons but also appears in migrating neural crest cells (Garcia-Gutierrez et al., 2014). Therefore, overexpression of Ptn abrogates crest migration. This suggests that *Brd2* is required for crest migration, and in fact, knockdown of *Brd2* or interfering with it by other means, also leads to migration arrest.

Two intriguing aspects are linked to the *Brd2*-Ptn interaction. First of them is that Ptn selectively interacts with *Brd2* among the different BET proteins (Garcia-Gutierrez et al., 2014). This occurs because of the presence of an exclusive acidic domain in *Brd2*, placed close to the dimerization motif B (**Figure 1**). Then, Ptn seems to interfere with *Brd2* activity by interfering with proper *Brd2* conformation. In relation to this it should be mentioned that, similar to *Brd2*, overexpression of other BET members also leads to impaired neuronal differentiation (Garcia-Gutierrez et al., 2014), rising the question of how selective interaction of Ptn with one BET member unbalance the cell toward differentiation. In this regard it is important to indicate that it has been shown that half the dose of single BET proteins in the cell has an impact in cell proliferation (Shang et al., 2009) and, indeed, rather than completely blocking all BET pool in the cell, partially interfering with BET proteins through selective interaction with a single member, better fits with a role of Ptn as a modulator. The second intriguing aspect of the *Brd2*-Ptn interaction is that the *Brd2* acidic region seems not to be conserved along

vertebrates. In fact, *Brd2* protein from lower vertebrates naturally lacks of the acidic region (**Figure 1**). Similarly, mouse Brd3 and Brd4 naturally lack this acidic region, and these proteins do not interact with Ptn (Garcia-Gutierrez et al., 2014). Whether Ptn homologs in lower vertebrates interact with the corresponding *Brd2* homolog has not been investigated. However, since deletion of just the 12-amino acids acidic stretch in mouse *Brd2* completely abolishes interaction, it is reasonable to anticipate that *Brd2* do not interact with Ptn in lower vertebrates. Does it mean that Ptn is not a modulator of *Brd2* in lower vertebrates? This is foreseeable; however, it has been reported *Mdk*-mediated enhanced neurogenesis in Xenopus (reviewed in Muramatsu, 2011), pointing to a role for Ptn/*Mdk* in neuronal differentiation also in lower vertebrates. It is quite probable that functions of Ptn/*Mdk* in neurogenesis are not restricted to antagonizing *Brd2*, but it is tempting to speculate that interaction with a BET member, and subsequently modulation of the balance between proliferation and differentiation, rises in higher vertebrates for a more accurate and fine tuning of neurogenesis. Indeed, neurogenesis proceeds in different ways in lower and higher vertebrates. In frogs and fishes, the so-called primary neurogenesis takes place in the neuroectoderm before closing of the neural tube, while in higher vertebrates the neural tube initially closes and then progenitors accumulate before starting neurogenesis. Thus, evolutionary differences, such as the ability of *Brd2* to interact with Ptn in higher vertebrates, should account for the different ways neurogenesis proceeds in both vertebrate groups.

Ptn and *Mdk* have been purified from different sources and tested as treatments in cells and model animals of disease (reviewed in Muramatsu, 2011). The therapeutic value of these proteins for treating neurodegenerative diseases, peripheral nerve injury and cancer has been emphasized according to previous reported Ptn/*Mdk* roles (Muramatsu, 2011). Ptn and *Mdk* are overexpressed in most studied cancers. Both transforming and angiogenic activities have been demonstrated for these proteins, and interfering with their expression has resulted in tumor arrest (reviewed in Muramatsu, 2011). As mentioned, inhibiting BET proteins has also been proved to be beneficial for cancer suppression in a variety of models (reviewed in Shi and Vakoc, 2014). Paradoxically, Ptn is involved in cancer at the same time that is able to enhance differentiation. Probably Ptn dose and the involved signaling pathway are critical aspects accounting for these apparent contradictory roles/effects. In Ptn/*Mdk* associated cancers, these proteins are highly expressed in the corresponding organs; therefore, the effects of Ptn/*Mdk* overexpression in tumor progression are not incompatible with a role of Ptn/*Mdk*, at physiological doses, in neuronal differentiation. Most reports have revealed Brd4 involvement in cancer progression. However, *Brd2*-associated lymphomas have also been described (reviewed in Belkina and Denis, 2012). Thus, despite the reported link between Ptn/*Mdk* and cancer, it is tempting to speculate whether certain *Brd2*-related cancers could be successfully arrested by treatment with these proteins. In sum, in addition to the many therapeutic properties formerly attributed to Ptn for the treatment of neurodegenerative diseases, nerve injury and cancer, Ptn emerges as an interesting molecule to be included in differentiation protocols for cell therapies in regenerative medicine, and remarkably, Ptn adds to the list of BET inhibitors, potentially useful for treatment of specific *Brd2*-associated cancers.

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Pablo Garcia-Gutierrez, Mario Garcia-Dominguez*

Stem Cells Department, Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER) (Consejo Superior de Investigaciones Cientificas (CSIC), Junta de Andalucía, Universidad de Sevilla, Universidad Pablo de Olavide). Av. Americo Vespucio s/n, 41092 Seville, Spain

***Correspondence to:** Mario Garcia-Dominguez, Ph.D., mario.garcia@cabimer.es.*
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