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REVIEW

The retinoblastoma gene is involved in multiple aspects of stem cell biology

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Genetic programs controlling self-renewal and multipotentiality of stem cells have overlapping pathways with cell cycle regulation. Components of cell cycle machinery can play a key role in regulating stem cell self-renewal, proliferation, differentiation and aging. Among the negative regulators of cell cycle progression, the RB family members play a prominent role in controlling several aspects of stem cell biology. Stem cells contribute to tissue homeostasis and must have molecular mechanisms that prevent senescence and hold 'stemness'. RB can induce senescence-associated changes in gene expression and its activity is downregulated in stem cells to preserve self-renewal. Several reports evidenced that RB could play a role in lineage specification of several types of stem cells. RB has a role in myogenesis as well as in cardiogenesis. These effects are not only related to its role in suppressing E2F-responsive genes but also to its ability to modulate the activity of tissue-specific transcription factors. RB is also involved in adipogenesis through a strict control of lineage commitment and differentiation of adipocytes as well in determining the switch between brown and white adipocytes. Also, hematopoietic progenitor cells utilize the RB pathway to modulate cell commitment and differentiation. In this review, we will also discuss the role of the other two RB family members: Rb2/p130 and p107 showing that they have both specific and overlapping functions with RB gene.
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Introduction

Stem cells within normal tissues are defined by common characteristics: self-renewal property to maintain the stem cell pool over the time; regulation of stem cell number through a strict balance between cell proliferation, cell differentiation and cell death; ability to give rise a broad range of differentiated cells. The genetic programs controlling the stem cell state or stemness (self-renewal, multipotentiality) are strictly linked to regulation of cell cycle (Morrison *et al*., 1997; Edlund and Jessell, 1999; Galderisi *et al*., 2003, 2006). In fact, to hold self-renewal property, a stem cell has to bypass cell cycle exit and undergo cell division in which one of or both of the resulting daughter cells remain undifferentiated and give rise to another stem cell. Alternatively, a stem cell can rest in quiescent state and then start to proliferate re-entering into cell cycle when extrinsic factors induce cell proliferation to replenish the pool of stem cells (Morrison *et al*., 1997; Edlund and Jessell, 1999; Galderisi *et al*., 2003, 2006).

Mitotic quiescence is a typical characteristic of stem cells and is a temporary exit from cell cycle. This event must be carefully controlled since it is distinct from permanent cell cycle exit. The latter represents the fundamental step to induce a novel program of gene expression leading to the elaboration of specialized phenotype (cell commitment and differentiation) or alternatively to trigger senescence. It is then clear that components of cell cycle machinery can play a key role in regulation of stem cell self-renewal, proliferation, differentiation and aging.

Several factors control cell cycle progression. The players in this scenario are holoenzymes composed of regulatory (cyclin) and catalytic cyclin-dependent kinase subunits (CDKs). CDKs are initially activated through a series of steps, beginning with the association with a cyclin subunit followed by phosphorylation/dephosphorylation of specific amino acids.

The G_1/S transition is the key step for cell cycle progression and is controlled by D-type cyclins/CDK4, D-type cyclins/CDK6, which act in mid G_1, and by cyclin E/CDK2, which operates in late G_1 (Kasten and Giordano, 1998; Stiegler *et al*., 1998; Harbour *et al*., 1999; Sherr and Roberts, 1999; Watanabe *et al*., 1999; Pucci *et al*., 2000).

One key substrate of CDKs/cyclins is the nuclear tumour suppressor pRb (and its related proteins pRb2/p130 and p107), which is phosphorylated on serine and threonine residues during G1 phase. pRb phosporylation results in the liberation of E2F factors, whose activity is required for entry into S phase (Kasten and Giordano, 1998; Stiegler *et al*., 1998; Harbour *et al*., 1999; Sherr and Roberts, 1999; Watanabe *et al*., 1999; Pucci *et al*., 2000).

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Two classes of molecules can bind to CDKs and inhibit their kinase activity, by arresting cells in G_1. One class includes $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$ and $p19^{INK4d}$, all of which contain characteristic fourfold ankyrin repeats. The second group of CDK inhibitors includes $p21^{Cip1}$, $p27^{Kip1}$ and $p57^{kip2}$. The Ink4 proteins specifically interact with CDK4 and CDK6 and impair their interaction with D-type cyclins, whereas Cip/Kip molecules act on cyclin/CDK by forming a ternary complex (Figure 1) (Stiegler *et al*., 1998; Harbour *et al*., 1999; Sherr and Roberts, 1999; Watanabe *et al*., 1999; Pucci *et al*., 2000).

Among the negative regulators of cell cycle progression the RB family members (mainly RB) play a prominent role in regulating several aspects of stem cell biology (Figure 1).

First evidences came from studies on knockout (k.o.) animal models showing that the silencing of these genes could greatly affect organism development, suggesting that impairment of tissue homeostasis in several organs could be ascribed also to altered functionality of stem cell compartments.

In mouse embryos that lack both copies of the RB gene, dividing neural precursor cells are found outside the normal neurogenic regions in both the central and peripheral nervous systems. Many of the ectopically dividing cells die by apoptosis; moreover, the expression of several neural differentiation markers is greatly reduced (Clarke *et al*., 1992; Jacks *et al*., 1992; Lee *et al*., 1992, 1994). Mice lacking RB show several defects also in hematopoiesis, such as reduced formation of hepatic blood islands and an increased percentage of immature nucleated erythroid cells. In addition, myogenesis appears greatly impaired (Clarke *et al*., 1992; Jacks *et al*., 1992; Lee *et al*., 1992, 1994).

Mice lacking either pRb2/p130 or p107 in a mixed 129/Sv:C57Bl/6J genetic background exhibited no overt phenotype; furthermore, they were viable and fertile (Lee *et al*., 1994; Cobrinik *et al*., 1996; Herrera *et al*., 1996; Hurford *et al*., 1997). Embryos lacking both pRb2/p130 and p107 died *in utero* 2 days earlier than RB-deficient embryos and exhibited apoptosis in the liver and central nervous system, suggesting some redundancy in function (Cobrinik *et al*., 1996; Lee *et al*., 1996). However, in a Balb/CJ genetic background the knockdown of pRB2/p130 produced embryonic lethality associated with increased cellular proliferation and apoptosis in the neural tube (LeCouter *et al*., 1998). The myocardium of Rb2/p130 k.o. embryos was abnormally thin and resembled an earlier staged two-chambered heart consisting of the bulbus cordis and the ventricular chamber (LeCouter *et al*., 1998).

Stemness and senescence

Embryonic stem cells

Embryonic stem cells (ESCs) have a peculiar cell cycle. Their cell division rates are very short and have a

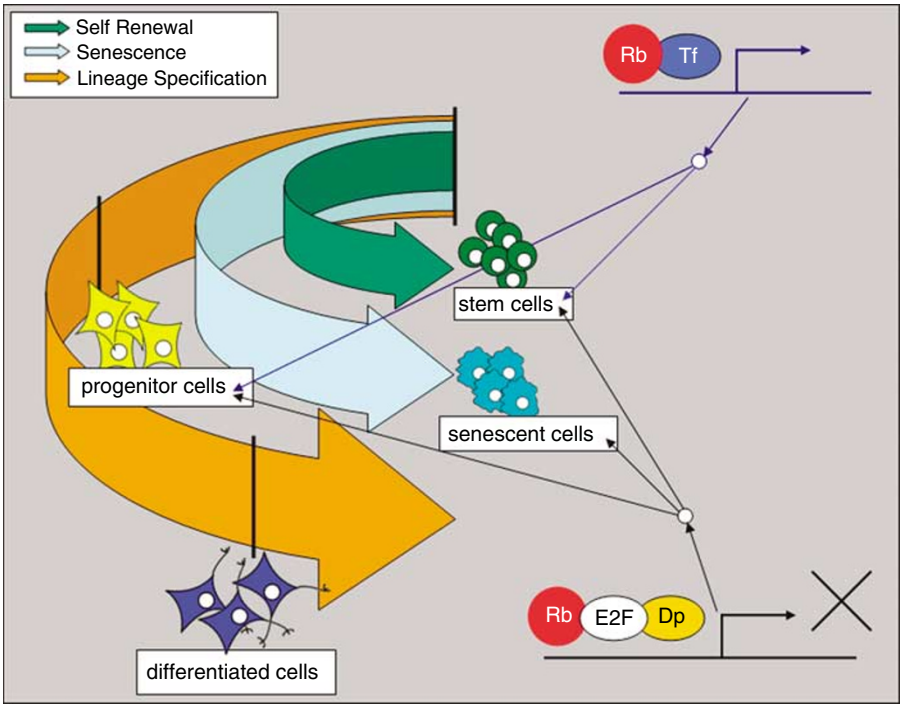

Figure 1 Stem cells biology and RB. Stem cells can undergo self-renewal, be committed toward restricted progenitors, which in turn differentiate into mature cells, or can become senescent cells. pRb can bind the E2F transactivation domain and directly block E2F activity. In addition, pRb can directly activate tissue-specific transcription factors. The inhibition of E2F-responsive genes can negatively affect stem cell self-renewal and promote senescence. Moreover, cell cycle exit, following inhibition of E2Fs can contribute to lineage specification and/or cell differentiation. The latter events are also promoted by interaction of pRb with tissue-specific transcription factors (Tf).

mechanism of CDK regulation that is different from that observed in other cell types. Proliferating ESCs have a very short G_1 phase (truncated G_1) and the great majority of cells are in S phase. The CDKI, such as p27, p21 and p16 are inactivated and only the cyclin B1/CDK1 complex has the typical periodicity. In addition, the pRb and p107 proteins are hyperphosphorylated and inactive. Consequently, E2F-responsive genes are transcribed independently of cell cycle progression (Stead *et al*., 2002; White *et al*., 2005). This unusual regulation of cell cycle is fundamental to hold 'stemness' avoiding cell specification and differentiation. In fact, changes in cell cycle during ESC differentiation are associated with the establishment of cell cycle-regulated CDK activities. White *et al*. (2005) evidenced that following induction of ESC differentiation the expression of cyclin A2 and E1 became cell cycle regulated, this in turn induced a regulation of CDK2 activity. Both CDKI and RB family members contribute to control CDK–cyclin complexes (White *et al*., 2005). This is a prerequisite to induce cell specification and cell differentiation. In fact, loss of pluripotency and stemness is associated with decreased CDK activity and activation of RB pathway that determines a periodicity in transcription of E2F-dependent genes such as cyclin E1 (White *et al*., 2005).

Regulation of cell cycle in ESCs raises the question: is self-renewal mechanistically coupled to cell division? Sage *et al*. (2000) evidenced that disruption of the RB family members in mouse embryonic fibroblasts (MEFs) induces loss of G_1 control and immortalization (Sage *et al*., 2000). Similar results were obtained by Dannenberg *et al*. (2000). They showed that MEFs with ablation of RB family members were completely insensitive to senescence-inducing signals and have a strong increase in their proliferation rate. In addition, these cells have a deregulated G_1 phase and become immortal (Dannenberg *et al*., 2000).

All these results suggest that there could be a link between cell cycle regulation and self-renewal. In this scenario, RB family members are associated with the loss of stemness.

Columella stem cells

In plants at the root apex there are stem cell compartments, called meristems that supply cells for organs and tissues forming the plant body. In roots the meristem is composed of columella stem cells that give rise to the several layers of differentiated cells containing amyloplasts (starch-storage organelles). In plants it has been identified a RB-related gene that regulates cell cycle progression as observed in animals. Silencing this gene by RNA-interference construct into *Arabidopsis thaliana* resulted in ectopic layers of stem cells (Wildwater *et al*., 2005). This study further suggests that pRb can negatively regulate stem cell self-renewal. According to these studies, RB could play a key role in regulating stem cell biology, since its activity appears to be well preserved in evolutionary divergent organisms.

Small intestine crypt stem cells

The inactivation of RB may be a key event to promote stem cell proliferation. In fact, Guo *et al*. (2005) demonstrated that in prototype small intestine crypt stem cells the activity of RB is regulated at multiple levels. The acute loss of intestinal regions triggers mitogenic signals to the intestinal crypt cells to grow taller villi and deepen the crypts in order to increase the area of remaining absorptive and digestive mucosa. Among the different mitogens, the EGF-mediated signaling appears to play a major role in this process (Guo *et al*., 2005). The treatment of RIEC-6 cell cultures (a model of small intestine crypt stem cells) with EGF induces a sustained proliferation that is associated with a heavy pRb phosphorylation. Usually, mitogens induce pRb phosporylation and subsequent inactivation through cyclin D/CDK4-6 and later through cyclin A/CDK2. In the RIEC-6 cells the EGF treatment induces an early pRb phosphorylation that is CDK independent. Several evidences suggest that ERK1/2 binds to pRb and causes its rapid phosphorylation (Guo *et al*., 2005).

RB and senescence

Cellular senescence is a fundamental tumor-suppressor mechanism. In fact, senescence is an irreversible growth arrest that is induced by DNA damage, oxidative stresses, imbalance in gene-expression and other cell-harmful stimuli. On the other hand, senescence can contribute to aging and age-related diseases (Campisi, 2001). Stem cells contribute to tissue homeostasis during the life-time, it follows that stem cells must have a molecular mechanism that prevents senescence by inhibiting the main senescence-associated pathways, which are the p16-RB pathway, ARF-p53 pathway and telomere-length regulation (Campisi, 2001).

RB is typically activated during senescence and its enforced expression can induce senescence in some cell types (Narita *et al*., 2003). Moreover, in cultures of human fibroblast the expression of SV40 large T antigen, which binds and inactivates both p53 and pRb, can inhibit the replicative senescence (Narita *et al*., 2003). The group of Lowe showed that senescent human fibroblasts accumulate a distinct heterochromatin structures, called senescence-associated heterochromatic foci (SAHF) that impair transcription. In particular, heterochromatic-associated proteins and pRb are localized on E2F-responsive promoters in senescent but not in quiescent cells. They showed also that pRb is required for SAHF formation (Narita *et al*., 2003).

The above described studies suggest that RB can play a key role to induce senescence-associated changes in gene expression and that stem cells must carefully control its activity in order to preserve self-renewal properties.

RB and transformation of stem cells

Like stem cells, cancer cells are widely thought to proliferate indefinitely through cellular self-renewal

capacity. This raises the possibility that some of the clinical properties of tumor cells may be due to transformed stem cells, called cancer stem cells. In fact, if signal transduction pathways that control stem cell renewal, proliferation and differentiation become deregulated it is possible that stem cells undergo cellular transformation (Galderisi *et al*., 2006). It is noteworthy, that many human cancer cells have mutation in one or more components of p53- and RB pathways. In fact, the inactivation of these pathways confers escape from senescence growth arrest and resistance to growth-inhibitory signals (Campisi, 2001; Galderisi *et al*., 2006).

Mutations in the RB gene has been shown to be present regularly either in sporadic or inherited form of retinoblastoma, where deletions of RB locus are reported frequently and considered as essential steps in tumor pathogenesis (Paggi *et al*., 1996).

Recently, Seigel *et al*. (2005) hypothesized that the markedly increased frequency of secondary malignant neoplasms observed in some retinoblastoma tumors could be attributed to the persistence of cancer stem cells that escape chemotherapy and cause metastasis (Seigel *et al*., 2005). As evidence, they demonstrated that in these tumors there are small populations of cells showing some characteristics of cancer stem cells, such the expression of ABCG2, a cell surface antigen that is marker to identify stem cells in several types of cancer. The 'putative cancer stem cells', lacking RB gene, were immunoreactive to stem cell marker aldehyde dehydrogenase 1 (ALDH1), which is present in hematopoietic progenitor cells and to SCA-1, a mouse-specific stem cell antigen (Seigel *et al*., 2005).

The role of RB in cell cycle regulation, senescence and cell differentiation can explain how RB suppresses tumor growth. However, these properties of RB do not explain completely why loss of RB function leads to multiple genetic alterations and hence to cancer susceptibility (Zheng *et al*., 2002). Zheng *et al*. (2002) evidenced that in ESCs the inactivation of RB gene leads to genetic instability. To analyse the level of chromosome instability they used retroviruses carrying both positive and negative selectable markers that integrated into chromosomes in a random fashion. They measured the loss of these selectable markers (LOMs) in cell cultures of mouse ESCs. In cells originating from RB k.o. mice the LOMs was significantly higher than in wild-type cultures. They also evidenced that the LOM was due to larger deletion of chromosomal event and not to point mutations. They suggested that in RB-deficient cells there is an impairment of mitotic division and of chromosome segregation (Zheng *et al*., 2002).

Lineage specification and cell differentiation

RB and myogenesis

Generation of RB k.o. mice evidenced a key role of this gene in myogenesis. RB cooperates with the myogenic basic helix–loop–helix factor MyoD to activate muscle-specific gene expression (Novitch *et al*., 1996). In primary mouse fibroblasts deficient for RB the ectopic expression of MyoD induces an aberrant muscle differentiation with the absence of late skeletal differentiation markers (Novitch *et al*., 1996). Other studies demonstrated that RB has a role also during early phases of myogenesis in satellite myoblasts that are the reservoir of stem cells in postnatal skeletal muscles. Li *et al*. (2000) investigated the pathways responsible for choice between differentiation and growth in satellite stem cells by ectopically expressing dominant-negative proteins that were capable of impairing muscle differentiation. To this end, they fused a library of cDNAs from skeletal muscles with lysosomal protease cathepsin B. In this way, the fusion proteins were delivered to the lysosome compartment and inactivated. The library was transfected into cultures of satellite stem cells that upon serum depletion exit the cell cycle and differentiated. By selection from the transfected cells of the clones capable of cell cycle re-entry and continued growth following serum starvation the library was enriched for cDNA molecules whose dominant-negative inhibition impair myoblast terminal differentiation. Among the several genes selected with this screen they identify the RB gene demonstrating a fundamental role in stem cell specification and differentiation (Li *et al*., 2000).

RB and cardiogenesis

There is a general agreement on the idea that RB may cooperate with tissue-specific factors to modulate cell differentiation. This hypothesis is based on the observation that the cell cycle exit promoted by RB could be the starting event for reprogramming gene expression profile of cells. On the basis of this hypothesis, RB should have a negligible role in cardiogenesis, since, in contrast to skeletal muscle cells, cardiomyocyte progenitors start to differentiate before leaving the cell cycle (Papadimou *et al*., 2005). On the other hand, several observations suggest a role for RB in cardiogenesis: the RB k.o. mice die of heart failure due to a thinning of ventricular wall (Ziebold *et al*., 2001).

To clarify the role of RB during the early stages of cardiogenesis, Papadimou *et al*. (2005) compared the cardiocyte commitment and differentiation of ESCs from RB k.o. mice with those of cells obtained from wild-type animals. Cardiac differentiation of RB $-/-$ ES cells was delayed and evidenced a decreased expression of the earliest cardiac-specific transcription factors (Nkx2.5 and Mef2c). Of interest, ectopic expression of RB in ES-derived cardiac progenitors rescued cardiac cell differentiation of Rb $-/-$ ES cells. The role of RB in cardiogenesis appears to be beyond its ability to regulate cell cycle. The lack of RB in cardioblasts did not trigger uncontrolled cell growth rather affected cell specification and differentiation (Papadimou *et al*., 2005). Recent findings suggest that the role of RB in cardiogenesis is not related to suppression of E2F-responsive genes but to its ability to modulate the activity of cardiogenic factors, such as CMF1 and LEK1 (Papadimou *et al*., 2005).

RB and adipogenesis

Adipocyte differentiation is a well-defined process that can be recapitulated with murine 3T3 mesenchymal-like stem cells as a model system (Sparks *et al*., 1986; Cole *et al*., 2004). The differentiation process can be divided into three steps: (1) growth arrest; (2) cell commitment and reversible differentiation; (3) terminal differentiation (Wang and Scott, 1993). Growth arrest is not sufficient to induce adipocyte differentiation, the PPAR factors and the C/EBP transcription factors are important for lineage specification and differentiation. 3T3 cells do not differentiate *in vitro* following exogenus stimulation with adipogenesis-cytokine cocktails. Only ectopic expression of PPAR-Υ and C/EBP-α in 3T3 cells commit them to a preadipocyte status and can promote terminal differentiation (Classon *et al*., 2000; Hansen *et al*., 2004).

Classon *et al*. (2000) evidenced a striking effect of RB and p107/p130 k.o. mice on adipocyte differentiation. 3T3 cells from p107/p130 k.o. mice expressed endogenous c/EBP-α and became adipocytes upon stimulation. At the opposite, 3T3 cells from RB k.o. mice, like the wild-type cells, did not express C/EBP-α and were unresponsive to *in vitro* differentiation. Moreover, the ectopic expression of RB in wild-type 3T3 promoted differentiation, while p107 did not. Taken together, these results suggest that RB can have a role in modulating the activity of transcription factor C/EBP-α that is involved in adipocyte differentiation. The induction of cell differentiation does not rely, at least completely, on the ability of RB to induce cell cycle exit. In fact, Rb $-/-$ 3T3 cells can be stimulated to differentiate by exogeneous expression of PPAR-Υ and C/EBP-α. Thus, the role of RB in adipogenesis does not appear to be linked to its ability to repress E2F-responsive promoters (Classon *et al*., 2000).

The importance of RB in adipocyte lineage commitment and differentiation is further underscored by researches on ESCs (Hansen *et al*., 2004). Hansen *et al*. (2004) demonstrated that RB can determine the switch of white adipose tissue versus the brown one. In mammals, thermogenesis is finely regulated by brown and white adipocytes (Lowell and Spiegelman, 2000; Rosen *et al*., 2000). The former dissipate energy, while the latter are the body energy stores. Molecular pathways controlling the switch between these two types of adipocyte cells are fundamental for tissue and organ homeostasis.

Cultures of ESCs and embryonic fibroblasts from RB k.o. mice can differentiate into brown-like adipocytes showing their typical gene expression pattern and mitochondria content. This is surprising, since the studies of Classon evidenced that culture of 3T3 cells lacking RB cannot differentiate into white adipocytes. Together, these researches suggest that RB has an opposing effect in white and brown adipogenesis: being required for differentiation of white adipose cells but impairing the maturation of brown adipocytes even in response to strong inducers of adipogenesis. These opposite effects can be accomplished by RB interaction with C/EBP-α for white adipogenesis and inhibiting the expression of forkhead transcription factor FoxC2, which in turn induces the expression of UCP-1, a typical protein of brown adipocytes (Hansen *et al*., 2004). RB could impair brown adipogenesis also through inhibition of thyroid hormone receptors, which are involved in brown adipocyte differentiation (Hansen *et al*., 2004).

RB and hematopoiesis

Human bone marrow progenitor cells can differentiate into several blood cell types. This process of cell commitment and differentiation can be modulated by interactions among extrinsic signals and intrinsic factors, such as hematopoietic-specific transcription factors (Shivdasani and Orkin, 1996; Tenen *et al*., 1997). RB contributes to hematopoietic cell lineage and maturation through interaction with several hematopoietic transcription factors, such as NF-IL-6, PU1, ELF1 (Bergh *et al*., 1999). Bergh *et al*. (1999) demonstrated that RB can determine the choice between neutrophilic and monocytic commitment of CD34+ pluripotent cells. They observed that monocyte maturation is associated with a striking increase of hypophosphorylated pRb (Bergh *et al*., 1999). Moreover, in cultures of bone marrow progenitor cells, incubated with cytokines promoting monocyte differentiation, the treatment with antisense oligonucleotides targeted against RB reduced the number of monocyte-like colonies, and, at the same time, promoted neutrophilic lineage commitment (Bergh *et al*., 1999). The authors hypothesize a double role for RB: it can promote monocyte differentiation by interacting with C/EBP transcription factors and could suppress activation of neutrophilic-specific transcription factors. The effects of RB on lineage commitment and cell differentiation occur independently of its ability to control cell cycle. In hematopoietic cells the cell cycle exit appear to be accomplished mainly through negative regulation of E2F transcription factors by RB-related protein Rb2/p130 (Hoshikawa *et al*., 1998).

Comparison of the role of RB and related pocket proteins in stem cell biology

Data on the role of p107 and Rb2/p130 on stem cell biology are very scant. This is also due to the initial observations suggesting that RB family proteins have overlapping functions. However, several studies have evidenced functional differences among these proteins (Claudio *et al*., 1994; Hurford *et al*., 1997; Jori *et al*., 2004; Caputi *et al*., 2005). In this review, we will focus only the differences in regulation of stem cell biology.

RB proteins in mesenchymal stem cell biology

We studied the effects of RB and RB2/p130 overexpression both in uncommitted and neural committed mesenchymal stem cells (MSCs), to evaluate if the molecular pathways related to the retinoblastoma gene family could contribute to the regulation of MSC proliferation, differentiation and apoptosis (Jori *et al*., 2004, 2005).

The regulation of cell cycle progression by both RB and RB2/p130 was observed both in MSCs grown as uncommitted cells and in those committed toward neural phenotypes. These results suggest that RB and RB2 have overlapping activities in cell cycle regulation. In addition, while the antiproliferative activity of RB is mainly HDAC-independent the cell growth arrest induced by pRb2/p130 relies, at least in part, on HDAC-related pathways (Jori *et al*., 2004, 2005).

While cell cycle regulation appears to be a general aspect of RB family biological function, the modulation of differentiation and apoptosis is strictly dependent on cellular context. In uncommitted MSCs, both RB and RB2/p130 did not affect the apoptotic process. On the contrary, while RB protected differentiating MSCs from apoptosis, RB2/p130 induced an increase in apoptosis compared to controls. The effects of both RB and RB2/p130 on programmed cell death appeared to be HDAC independent (Jori *et al*., 2004, 2005).

Why does pRb demonstrate antiapoptotic activities while pRb2/p130 triggers cell death? An in-depth study on molecular pathways triggered by these genes is highly desirable to answer this question. Nevertheless, we might hypothesize that these phenomena are at least partially due to the fact that pRb can protect cells from death since it has the ability to bind and repress all the E2F transcription factors, including E2F1, which has proapoptotic properties. On the contrary, pRb2/p130 binds exclusively to E2F4 and E2F5 (Paggi *et al*., 1996; Stiegler *et al*., 1998).

We evaluated also whether the RB family members could contribute to neural commitment and/or differentiation. Our results demonstrated that RB family members do not have the ability to commit MSCs toward neural phenotypes. On the other hand, once the commitment/differentiation process has been triggered by NIM treatment, the RB and RB2/P130 genes contribute to the neural differentiation process (Jori *et al*., 2004, 2005).

Several authors have proposed that pRb and pRb2/p130 could have almost completely overlapping activities (Mulligan and Jacks, 1998). The effects that the ectopic expression of these genes had on the apoptosis machinery of differentiating MSCs and also on the phenotype of differentiating neurons demonstrated, instead, that even if the RB genes work everywhere to block cell cycle progression, each of them could preferentially act on specific biological functions.

RB family proteins in neural stem cell biology

The mechanisms that govern commitment and differentiation of the cells of the nervous system begin to be elucidated; however, how extrinsic and intrinsic components are related remains still poorly understood. To investigate this issue, we used neural stem cells (NSCs), obtained from the subventricular zone of newborn rats brains; we overexpressed the genes of the retinoblastoma family RB and RB2/p130, which have been shown to play an important role during nerve cells maturation, in NSCs induced to differentiate *in vitro* under neurogenic or gliogenic culture conditions (Jori *et al*., 2006).

The analysis of NSCs differentiation in cultures overexpressing RB and RB2/p130 revealed that these genes play an important role in the critical step in which the cell-fate of multipotent precursors is determined. Indeed, they promoted significant variations of the percentage of differentiated neurons, astrocytes and oligodendrocytes, as evidenced by immunofluorescent stainings performed to identify cell types in culture and by the expression levels of cell-type-specific molecular markers (Jori *et al*., 2006).

However, it seems that these genes are not able to influence *per se* the fate of NSCs, but rather cooperate with the extracellular signals that promote the specification of a given cell type (Jori *et al*., 2006).

The study of Vanderluit *et al*. (2004) further demonstrated that components of RB family could regulate the 'life' of NSCs. They showed that p107 play a role in the expansion of neural stem population obtained from embryonic and adult mammalian brains. *In vitro*, they observed an enhanced number of primary neurospheres from p107 k.o. mice compared with controls and, *in vivo*, they detected a larger population of slowly dividing precursors cell in adult p107 k.o. mice compared with wild-type animals. They hypothesized that p107 controls self-renewing stem cell division. In fact, the higher numbers of secondary neurospheres generated from p107-null NSC primary cultures is indicative of a high rate of self-renewing. These studies demonstrated a novel role for p107 that is distinct from RB and RB2/p130 (Vanderluit *et al*., 2004).

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References

Bergh G, Ehinger M, Olsson I, Jacobsen SE, Gullberg U. (1999). *Blood* **94**: 1971–1978.

Campisi J. (2001). *Trends Cell Biol* **11**: S27–S31.

Caputi M, Russo G, Esposito V, Mancini A, Giordano A. (2005). *J Cell Physiol* **205**: 319–327.

Clarke AR, Maandag ER, van Roon M, van der Lugt NM, van der Valk M, Hooper ML *et al*. (1992). *Nature* **359**: 328–330.

Classon M, Kennedy BK, Mulloy R, Harlow E. (2000). *Proc Natl Acad Sci USA* **97**: 10826–10831.

Claudio PP, Howard CM, Baldi A, De Luca A, Fu Y, Condorelli G *et al*. (1994). *Cancer Res* **54**: 5556–5560.

Cobrinik D, Lee MH, Hannon G, Mulligan G, Bronson RT, Dyson N *et al*. (1996). *Genes Dev* **10**: 1633–1644.

Cole KA, Harmon AW, Harp JB, Patel YM. (2004). *Am J Physiol Cell Physiol* **286**: C349–C354.

Dannenberg JH, van Rossum A, Schuijff L, te Riele H. (2000). *Genes Dev* **14**: 3051–3064.
Edlund T, Jessell TM. (1999). *Cell* **96**: 211–224.
Galderisi U, Cipollaro M, Giordano A. (2006). *Cell Death Differ* **13**: 5–11.
Galderisi U, Jori FP, Giordano A. (2003). *Oncogene* **22**: 5208–5219.
Guo J, Sheng G, Warner BW. (2005). *J Biol Chem* **280**: 35992–35998.
Hansen JB, Jorgensen C, Petersen RK, Hallenborg P, De Matteis R, Boye HA *et al*. (2004). *Proc Natl Acad Sci USA* **101**: 4112–4117.
Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC. (1999). *Cell* **98**: 859–869.
Herrera RE, Sah VP, Williams BO, Makela TP, Weinberg RA, Jacks T. (1996). *Mol Cell Biol* **16**: 2402–2407.
Hoshikawa Y, Mori A, Amimoto K, Iwabe K, Hatakeyama M. (1998). *Proc Natl Acad Sci USA* **95**: 8574–8579.
Hurford Jr RK, Cobrinik D, Lee MH, Dyson N. (1997). *Genes Dev* **11**: 1447–1463.
Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, Weinberg RA. (1992). *Nature* **359**: 295–300.
Jori FP, Galderisi U, Napolitano MA, Cipollaro M, Cascino A, Giordano A *et al*. (2006) (in press).
Jori FP, Melone MA, Napolitano MA, Cipollaro M, Cascino A, Giordano A *et al*. (2005). *Cell Death Differ* **12**: 65–77.
Jori FP, Napolitano MA, Melone MA, Cipollaro M, Cascino A, Giordano A *et al*. (2004). *J Cell Physiol* **200**: 201–212.
Kasten MM, Giordano A. (1998). *Cell Death Differ* **5**: 132–140.
LeCouter JE, Kablar B, Whyte PF, Ying C, Rudnicki MA. (1998). *Development* **125**: 4669–4679.
Lee EY, Chang CY, Hu N, Wang YC, Lai CC, Herrup K *et al*. (1992). *Nature* **359**: 288–294.
Lee EY, Hu N, Yuan SS, Cox LA, Bradley A, Lee WH *et al*. (1994). *Genes Dev* **8**: 2008–2021.
Lee MH, Williams BO, Mulligan G, Mukai S, Bronson RT, Dyson N *et al*. (1996). *Genes Dev* **10**: 1621–1632.
Li FQ, Coonrod A, Horwitz M. (2000). *Mol Cell Biol* **20**: 5129–5139.
Lowell BB, Spiegelman BM. (2000). *Nature* **404**: 652–660.
Morrison SJ, Shah NM, Anderson DJ. (1997). *Cell* **88**: 287–298.
Mulligan G, Jacks T. (1998). *Trends Genet* **14**: 223–229.
Narita M, Nunez S, Heard E, Lin AW, Hearn SA, Spector DL *et al*. (2003). *Cell* **113**: 703–716.
Novitch BG, Mulligan GJ, Jacks T, Lassar AB. (1996). *J Cell Biol* **135**: 441–456.
Paggi MG, Baldi A, Bonetto F, Giordano A. (1996). *J Cell Biochem* **62**: 418–430.
Papadimou E, Menard C, Grey C, Puceat M. (2005). *EMBO J* **24**: 1750–1761.
Pucci B, Kasten M, Giordano A. (2000). *Neoplasia* **2**: 291–299.
Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. (2000). *Genes Dev* **14**: 1293–1307.
Sage J, Mulligan GJ, Attardi LD, Miller A, Chen S, Williams B *et al*. (2000). *Genes Dev* **14**: 3037–3050.
Seigel GM, Campbell LM, Narayan M, Gonzalez-Fernandez F. (2005). *Mol Vis* **11**: 729–737.
Sherr CJ, Roberts JM. (1999). *Genes Dev* **13**: 1501–1512.
Shivdasani RA, Orkin SH. (1996). *Blood* **87**: 4025–4039.
Sparks RL, Seibel-Ross EI, Wier ML, Scott RE. (1986). *Cancer Res* **46**: 5312–5319.
Stead E, White J, Faast R, Conn S, Goldstone S, Rathjen J *et al*. (2002). *Oncogene* **21**: 8320–8333.
Stiegler P, Kasten M, Giordano A. (1998). *J Cell Biochem Suppl* **30–31**: 30–36.
Tenen DG, Hromas R, Licht JD, Zhang DE. (1997). *Blood* **90**: 489–519.
Vanderluit JL, Ferguson KL, Nikoletopoulou V, Parker M, Ruzhynsky V, Alexson T *et al*. (2004). *J Cell Biol* **166**: 853–863.
Wang H, Scott RE. (1993). *Cell Prolif* **26**: 55–66.
Watanabe Y, Watanabe T, Kitagawa M, Taya Y, Nakayama K, Motoyama N. (1999). *Brain Res* **842**: 342–350.
White J, Stead E, Faast R, Conn S, Cartwright P, Dalton S. (2005). *Mol Biol Cell* **16**: 2018–2027.
Wildwater M, Campilho A, Perez-Perez JM, Heidstra R, Blilou I, Korthout H *et al*. (2005). *Cell* **123**: 1337–1349.
Zheng L, Flesken-Nikitin A, Chen PL, Lee WH. (2002). *Cancer Res* **62**: 2498–2502.
Ziebold U, Reza T, Caron A, Lees JA. (2001). *Genes Dev* **15**: 386–391.