Exp Oncol 2017
39, 1, 12–16

POINT OF VIEW

Experimental ONCOLOGY

DO MRPS18-2 AND RB PROTEINS COOPERATE TO CONTROL CELL STEMNESS AND DIFFERENTIATION, PREVENTING CANCER DEVELOPMENT?

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In childhood tumors, including retinoblastoma, osteosarcoma, and neuroblastoma, the RB-E2F1 pathway is inactivated, as a rule. These tumors arise from precursor cells that fail to undergo the terminal differentiation. Noteworthy, the *RB1*-encoded protein (RB) does not control the cell cycle in embryonic stem cells. It has not been yet well understood how RB controls cell stemness and differentiation. The question arises why "inactive" RB is required for the survival and stemness of cells? Recently, we have found that overexpression of the RB-binding protein MRPS18-2 (S18-2) in primary fibroblasts leads to their immortalization, which is accompanied by the induction of embryonic stem cell markers and, eventually, malignant transformation. We suggest that cell stemness may be associated with high expression levels of both proteins, RB and S18-2. There must be a strict regulation of the expression levels of S18-2 and RB during embryogenesis. Disturbances in the expression of these proteins would lead to the abnormalities in development. We think that the S18-2 protein, together with the RB, plays a crucial role in the control on cell stemness and differentiation. We hope to uncover the new mechanisms of the cell fate determination. The S18-2 may serve as a new target for anticancer medicines, which will help to improve human health.

Key Words: **mitochondrial ribosomal protein MRPS18-2, retinoblastoma protein RB, stem cells, differentiation, cancer development, childhood tumors.**

Retinoblastoma is a sporadic or hereditary childhood tumor that arises in the retina. Retinoblastoma is usually diagnosed at an early age, such as before the age of two years in cases of hereditary tumor. The age-specific window of retinoblastoma growth suggests that tumor formation depends on the proliferation of cells transiently found in the retina (developing retina) [1]. The main genetic background for retinoblastoma is inactivation (mostly deletions) of the *RB1* gene [2]. The *RB1* gene is the first tumor suppressor gene that was cloned and its deletion was connected with tumor growth.

The *RB1*-encoded protein (RB, NP_000312) is a phosphoprotein expressed uniformly in all tissues. The RB is a major regulator of the cell cycle, blocking entry into the S-phase by binding to E2F1. The E2F1 (NP_005216) and several other members of E2F family are transcription factors, which transactivate genes required for the entry into the S-phase. RB protein binds to E2F1 and inhibits the transcriptional activity of the latter (Fig. 1) [3, 4]. In case of the RB phosphorylation, the E2F1 is released from the protein complex and performs its function. Hence, loss of *RB1* leads to uncontrolled cell proliferation. Most probably, this explains also retinoblastoma development.

Importantly, even if the patients with retinoblastoma are cured, they often develop osteosarcomas and melanomas later in life. Osteosarcomas, tumors of the skeleton, arise from the bone-forming cells; usually in the long bones and in the age window of 10–15 years. Genetic conditions of osteosarcomas are similar to retinoblastomas — mainly deletions of *RB1* gene, i.e. inactivation of the RB-E2F1 pathway [5].

Melanomas develop from the proliferating melanoblasts. In melanomas the RB-E2F1 pathway is also inactivated, mainly by alterations in the *INK* locus on chromosome 9 [6, 7]. This locus encodes two proteins, CDKN2A (p16, NP_000068) and CDKN2B (p15, NP_004927); they bind to and inhibit the cyclin-dependent kinases CDK4 (NP_000066) and CDK6 (NP_001138778). The CDKs phosphorylate RB protein. It was shown that promoter regions of the *CDKN2A* and *CDKN2B* genes are heavily methylated in melanomas; consequently, levels of encoded proteins drop dramatically. Phosphorylation of RB is not inhibited then, resulting in the S-phase progression (see Fig. 1).

Noteworthy, the E2F1-RB pathway is not functional also in one of the most dangerous childhood tumors, neuroblastoma. Neuroblastoma, sometimes called the embryonic tumor, arises from proliferating neuroblasts. It was shown that the MYCN (NP_001280157) protein is overexpressed in neuroblastomas. MYCN induces transactivation of the *ID2* gene. As a result, the highly expressed ID2 (NP_002157) protein competes with the E2F1 for the binding to RB. As was described above, the free E2F1 promotes cell division [8, 9].

Apart from the inactivation of the RB-E2F1 pathway, and, presumably, enhanced proliferation of cancerous cells, what are the other common features of these four tumor types?

Actually, all of the above-mentioned tumors arise from the partially differentiated neural crest stem cells. Thus, retinoblastoma in the eye arises due to proliferation of retinoblasts (retinal progenitor cells), which

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Abbreviations used: RB – *RB1*-encoded protein; S18-2 – RB-binding protein MRPS18-2.

should otherwise specialize into optical nerve cells. In other words, the terminal differentiation of retinoblasts is inhibited.

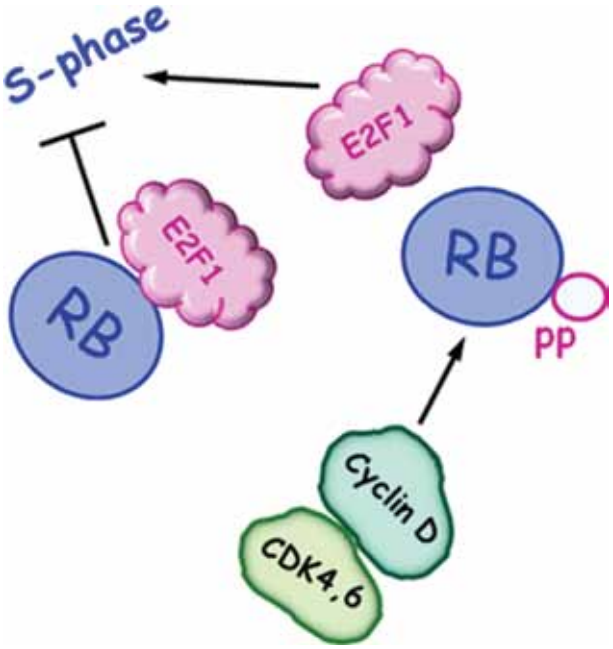

Fig. 1. The RB protein is a major regulator of the cell cycle. RB blocks entry into the S-phase by binding to E2F1. The cyclin-dependent kinases CDK4 and CDK6 phosphorylate RB, freeing the E2F1 from a protein complex. This results in S-phase progression

Moreover, osteosarcoma results from the proliferating osteoblasts. Normally, osteoblasts should undergo terminal differentiation into osteocytes. Melanoma cells are the proliferating undifferentiated melanoblasts (melanocyte precursors). Neuroblastoma is formed by proliferating neuroblast precursor cells. Upon the normal developmental process, the neuroblast precursor cells are destined to differentiate into neurons or die by apoptosis. Hence, all these tumors arise from precursor cells that fail to undergo the terminal stage of differentiation (summarized in Fig. 2). What can be a reason for inhibition of differentiation, in addition to inactivation of the RB-E2F1 pathway, supporting cell division?

Yet unexplainably, the RB protein in embryonic stem cells is present mainly in hypo- and hyperphosphorylated forms, i.e. not in protein complex with the E2F1 [10]. Hence, embryonic cell should proliferate upon such conditions. On the other hand, loss of *RB1* leads to embryonic lethality [11]. It is still not well understood how the RB is involved in control on cell stemness and differentiation (see [12] for review). A question arises why the "inactive" RB is required for the maintenance of cell stemness and normal development of a set of tissues?

We propose one of the possible explanations. Earlier, we have shown that RB binds to the human mitochondrial ribosomal protein MRPS18-2 (NP_054765, S18-2 in the text). This binding prevents RB-E2F1 complex formation, thus promoting S-phase entry (Fig. 3) [13, 14].

Moreover, overexpression of the S18-2 protein in primary rat embryonic fibroblasts leads to their immortalization with the induction of embryonic stem cell markers [15, 16]. Terminally differentiated primary rat skin fibroblasts underwent cell transformation upon ectopic expression of the S18-2 protein. The trans-

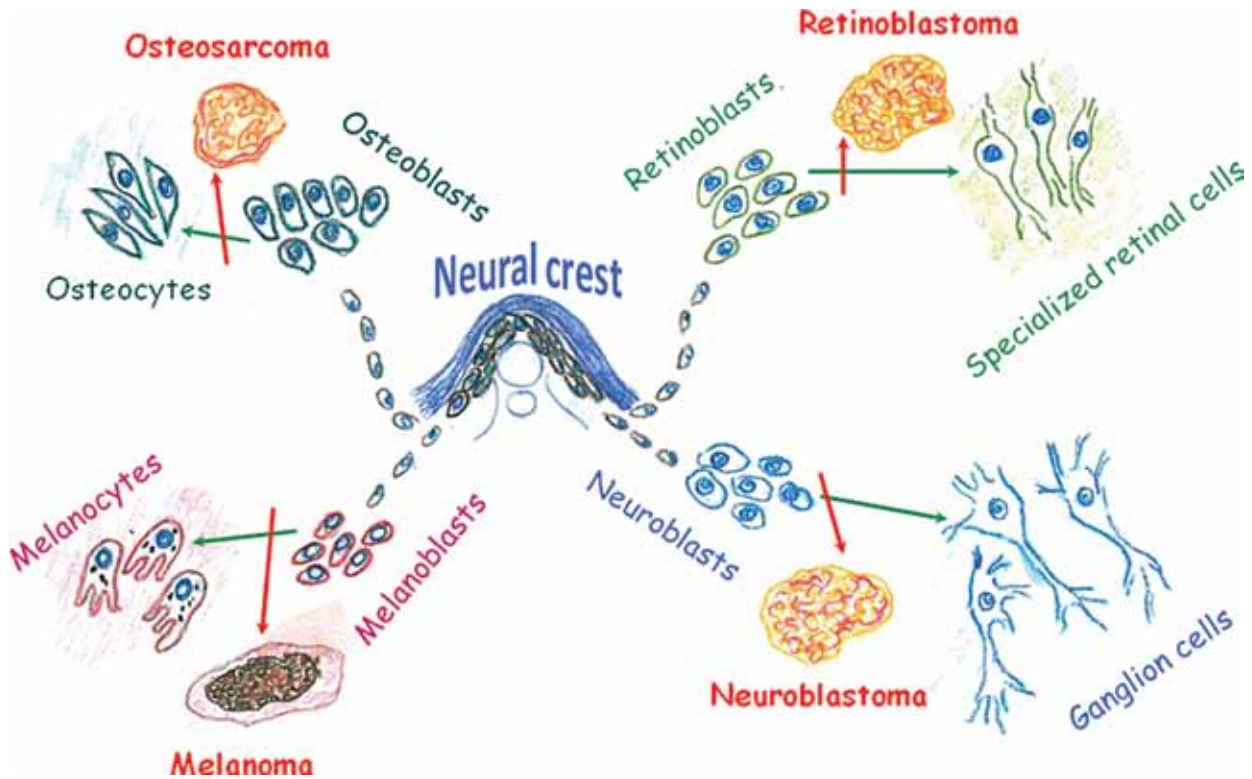

Fig. 2. Tumors arise from precursor cells that fail to undergo terminal differentiation. The migrating neural crest stem cells are shown in black. The migrating cells differentiate upon arrival to their organ of destination: bone (the upper left panel), skin (the lower left panel), retina (the upper right panel), and the sympathetic nervous system (the lower right panel). If the terminal differentiation of precursor cells is blocked, the tumor arises

formed cells showed increased telomerase activity, cell cycle disturbance, and chromosomal instability [17]. We concluded that the S18-2 is an oncoprotein and might be involved in carcinogenesis. We have shown recently that the S18-2 protein is expressed at high levels in endometrial cancers compared to hyperplasia and normal endometrium, along with the high level of the free E2F1 [18].

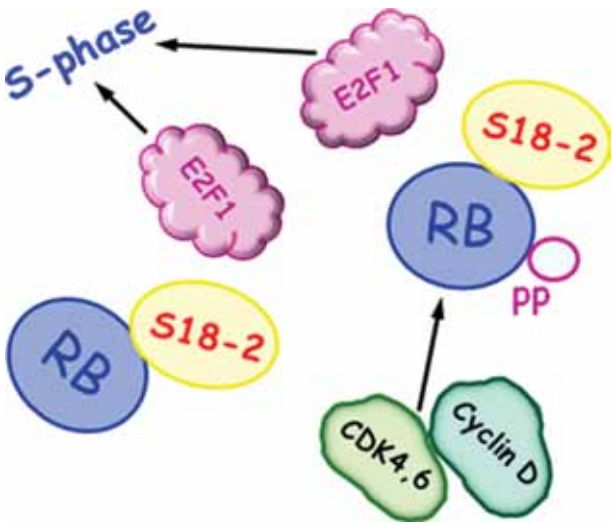

Fig. 3. Mitochondrial ribosomal protein S18-2 binds to RB, preventing the formation of RB-E2F1 complex, thus promoting S-phase entry

Our findings and analysis of the published microarray data showed the elevated expression of the S18-2 in stem and cancerous cells. Interestingly, levels of the *S18-2* are high in the EBV-transformed B-cells and transformed fibroblasts (Fig. 4, indicated by arrows). Besides this, heart, adrenal glands, and skeletal muscles showed the high *S18-2* levels. The data used for the analyses described in Fig. 4 were obtained from the GTEx Portal (*http://www.gtexportal.org/home*) on 27/01/2017.

Interestingly, upon the analysis of the S18-2 expression pattern in CCLE (Cancer cell line encyclopedia) at Broad Institute website (*https://portals.broadinstitute.org/ccle*), we noticed that the *S18-2* expression was quite low in cell lines derived from osteosarcomas, neuroblastomas, and chondrosarcomas. In melanoma cell lines the *S18-2* level was high (Fig. 5, indicated by arrows). The data used for the analyses described in Fig. 5 were obtained from the Broad-Novartis CCLE portal (*https://portals.broadinstitute.org/ccle*) on 27/01/2017. Noteworthy, in melanoma the RB-E2F1 pathway is inactivated not by *RB1* loss, i.e. the RB protein is present.

Unfortunately, not much data is reported, concerning the molecular mechanisms of chondrosarcoma development. Few authors reported both, loss of RB protein due to loss of heterozygosity of *RB1* gene [19], and also methylation of the CDKN2A promoter [20, 21].

The discussed above leads to a hypothesis that the RB and S18-2 proteins are involved together in the maintenance of cell stemness. Hence, the stem cells that could be terminally differentiated should express both, RB and S18-2 proteins.

The downregulation of levels or inhibition of the function by binding to other proteins of one or both proteins would lead to a failure in the differentiation of stem cells and, eventually, tumorigenesis. There must be a strict control on the expression of the S18-2 and RB: any disturbance in the expression (upregulation or downregulation) would result in abnormal proliferation and/or inhibition of differentiation (Fig. 6).

It is feasible to test the proposed hypothesis using, for example the *RB1* knockout mouse fibroblasts model. Another possibility is to work with a zebrafish model to knock down S18-2. Also, the sub-lines of cancer cells derived from retinoblastoma, osteosarcoma,

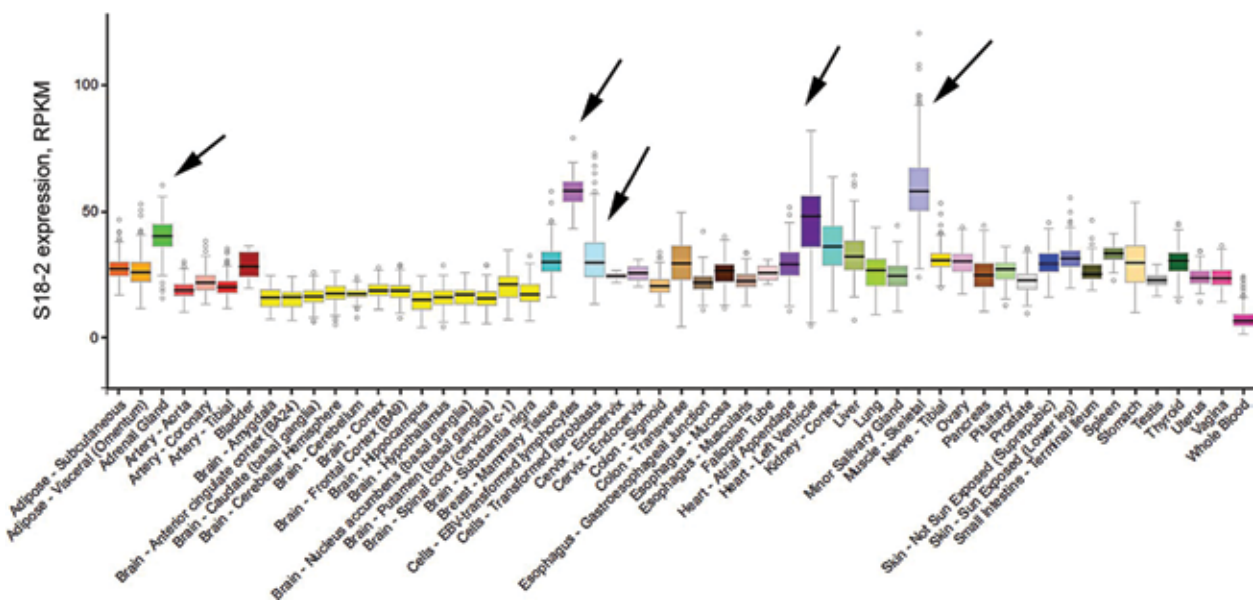

Fig. 4. Expression profile of the *S18-2* gene (Entrez Gene ID: 28973). The highest levels of the S18-2 were detected in adrenal gland, heart and skeletal muscles, as well as in transformed fibroblasts and EBV-transformed lymphocytes (indicated with arrows). RPKM – read per kilobase per million mapped reads, normalized to the gene length (see detailed description at *http://www.gtexportal.org/home*)

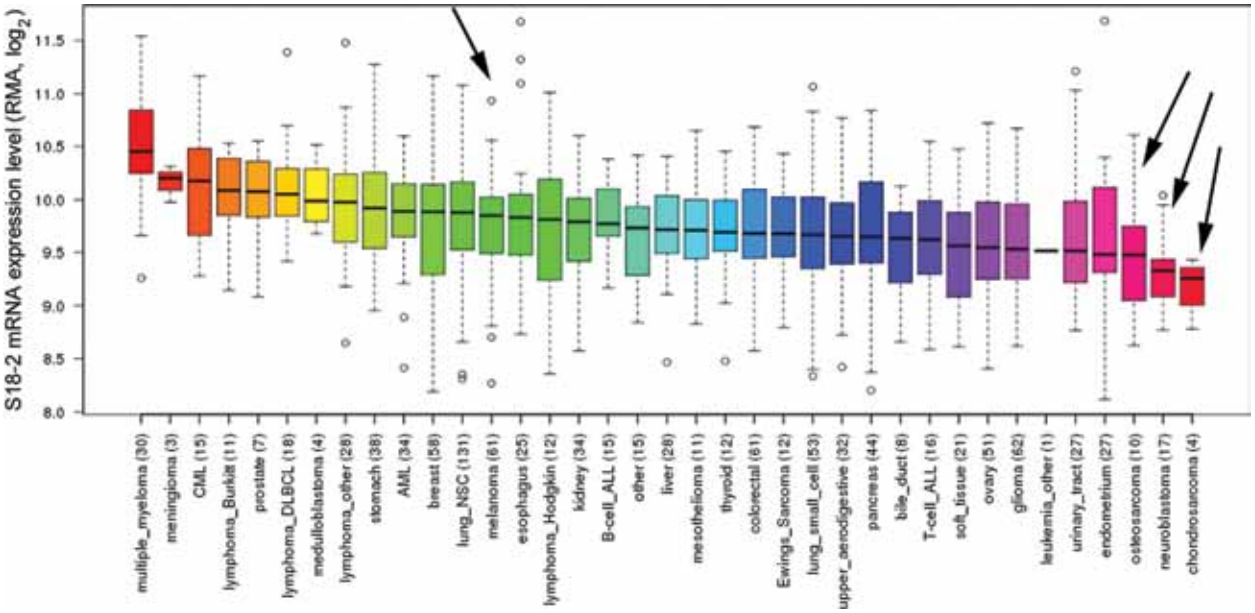

Fig. 5. Expression profile of the *S18-2* gene in immortalized cell lines (Entrez Gene ID: 28973). The lowest levels of the *S18-2* were detected in osteosarcoma, chondrosarcoma, and neuroblastoma cell lines (indicated with arrows, the right side of Fig. 5). RMA — Robust multichip averaging, relative units of the signal intensity (see detailed description at *https://portals.broadinstitute.org/ccle*)

melanoma, and neuroblastoma, expressing the exogenous S18-2 and RB at the high levels could be generated. The differentiation potential of the obtained cells and their tumorigenicity could be tested then.

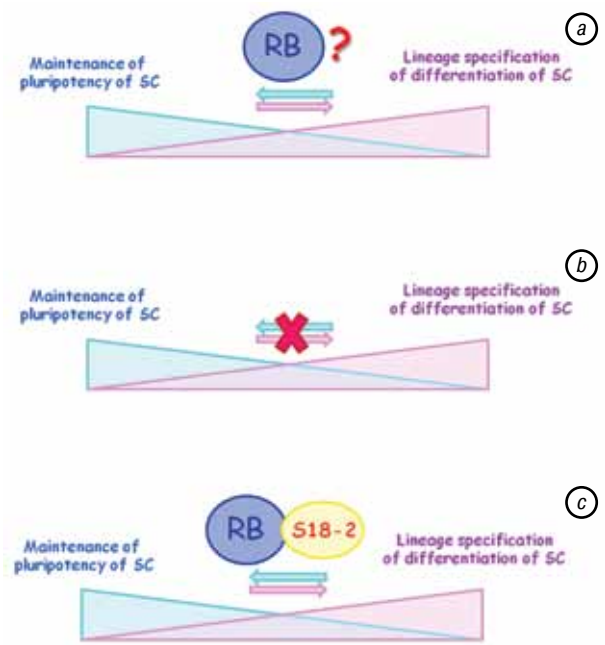

Fig. 6. RB controls cell stemness and differentiation. (*a*) RB controls cell stemness and differentiation, keeping the balance of these two processes. Mechanism of regulation is still not well understood. (*b*) Any disturbance in the RB expression would result in abnormal proliferation and/or inhibition of differentiation. (*c*) We hypothesize that RB and S18-2 proteins cooperate to maintain cell stemness. Hence, the stem cells could be characterized by the high expression levels of both, RB and S18-2 proteins. If cells loose RB (or S18-2), no terminal differentiation could be achieved. This leads to the tumor development then

Concluding, we think that the S18-2 protein, together with the RB, plays a crucial role in the control on cell stemness and differentiation. We hope to uncover the new mechanisms of the cell fate determination. The S18-2 may probably serve as a new target for anticancer medicines, which will help to improve human health.

ACKNOWLEDGMENTS

We thank Professor George Klein for the fruitful discussions on the proposed hypothesis.

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