SOX10-MITF pathway activity in melanoma cells

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Abstract

Melanoma is one of the most dangerous and lethal skin cancers, with a considerable metastatic potential and drug resistance. It involves a malignant transformation of melanocytes. The exact course of events in which melanocytes become melanoma cells remains unclear. Nevertheless, this process is said to be dependent on the occurrence of cells with the phenotype of progenitor cells – cells characterized by expression of proteins such as nestin, CD-133 or CD-271. The development of these cells and their survival were found to be potentially dependent on the neural crest stem cell transcription factor SOX10. This is just one of the possible roles of SOX10, which contributes to melanomagenesis by regulating the SOX10-MITF pathway, but also to melanoma cell survival, proliferation and metastasis formation. The aim of this review is to describe the broad influence of the SOX10-MITF pathway on melanoma cells.

Key words: melanoma, targeted therapy, vemurafenib, SOX10, BRAF, MITF.

Introduction

Rates of melanoma have been rising for at least 30 years. According to the World Health Organization, about 132 000 new melanoma cases were diagnosed in 2015. Survival rates depend mainly on the stage of melanoma when it is detected. However, there are a few additional factors to affect the patient's prognosis, such as genetic changes in the cancer cells and how well the cancer responds to treatment. Overall, 9% of patients die within 5 years after melanoma diagnosis.

The process of melanomagenesis, which comprises different steps leading to the progression from normal melanocytes to melanoma, may be triggered by a few risk factors. A major risk factor for most melanomas is excessive sun exposure. Moreover, tanning beds and sun lamps are very dangerous for people, as they provide an additional source of UV radiation. Also, a 20% increased risk was identified for regular alcohol consumers [1]. Overall, melanoma risk factors lead to damage of the mechanism which protects melanocytes from malignant transformation. This results in the development of new cells which have some qualities of melanocyte stem cells (MSCs). They are characterized by the expression of stem cell markers, e.g. nestin, CD-271 and CD-133 (more details are included in Table I).

The occurrence of stem cell markers is accompanied by a change in the metabolism of melanocytes. Unlike normal cells, whose proliferation is carefully controlled, these cells may undergo multiple divisions in an asymmetrical or symmetrical manner (the exact mechanisms by which

Table I. Characteristics of melanoma stem cell markers

Selected stem cell markers	Description	References
CD-271	Considered to be a predominant molecule in malignant melanoma and a crucial determinant of stem-like properties of melanoma cells such as colony formation and tumorigenicity	[2, 6, 8]
Nestin	An intermediate filament protein. Nestin is said to be a neuroectodermal stem cell marker. It was found that nestin expression could be reinduced after either tissue injury or melanomagenesis. It has a role in cell dynamics. Its development in melanoma cells potentially facilitates melanoma metastasis formation	[5, 39, 40, 43, 44]
CD-133 (Prominin-1)	A glioma and neural stem cell marker. It has a role in melanoma cell motility, metastatic and tumorigenic potential	[8, 27]

these cells divide have not been well characterized yet) [2]. Hence, it is possible that these cells play the role of seeds which bring about a fully developed tumor [3].

The results of numerous studies show that the activity of the SOX10-MITF (SOX10 = Sry-related HMG-box-10; MITF = microphthalmia-associated transcription factor) pathway is the reason why the expression of stem cell markers in melanocytes is possible [4–8]. Table I describes stem cell markers of great importance for melanomagenesis.

Numerous studies focus on the influence of SOX10 on stem cell marker expression. Flammiger *et al.* observed significantly higher expression of nestin in the presence of SOX10 [5]. A study by Shakhova *et al.* [6] showed that the suppression of SOX10 reduced the amount of CD-271+ tumor-initiating cells, which may constitute circumstantial evidence for a correlation between SOX10 and CD-271. CD-271 is a crucial protein for maintaining the hypothetical melanoma stem cell properties, including the capacity of self-renewal, resistance to chemotherapy and radiotherapy [1, 2]. Furthermore, recent work has revealed that the presence of another stem cell marker, CD-133$^+$, a molecule which is responsible for melanoma cell motility, metastatic and tumorigenic potential of cells, seems to be the consequence of previous CD-271 occurrence. Moreover, since CD-271 seems to be directly dependent on SOX10, CD-133 is potentially also dependent on the presence of SOX10 [1, 6–8].

This interdependence shows that SOX10 may constitute a crucial factor during the acquisition of the features of stem cells. Moreover, the SOX10-MITF pathway turned out to be involved in maintaining the proliferative and tumorigenic capacity of already existing melanoma cells, cell cycle regulation, expression of survival factors, metastasis formation and many other biochemical processes in melanoma cells. Hence, expanding our knowledge about the function of SOX10 and the SOX10-MITF pathway in melanoma cells may facilitate the development of novel melanoma target therapies. It could be particularly helpful in deleting the stem cell signature, which may result in the reduction of the tumor self-renewing potential [2, 4–8].

The purpose of this review is to describe how the SOX10-MITF pathway contributes to melanomagenesis, particularly to the development of melanoma cells with the stem cell phenotype. Since recent research revealed that SOX10 is involved in many processes in melanoma cells, another purpose of this review is to gather existing knowledge about the exact role of SOX10 in melanoma cell biology. Eventually, the last part of this review comprises a description of the role of SOX10 in vemurafenib resistance in melanoma. As vemurafenib is the most important targeted therapy for BRAF V600 mutation positive, unresectable or metastatic melanoma (about one-half of metastatic melanomas contain a specific mutation in the BRAF gene), new approaches are required to deal with resistance to vemurafenib. SOX10 seems to be very important in that context.

Cancer stem cells

More and more accepted theories state that cancer is a heterogeneous collection of cells which belongs to at least two distinct groups:

1. The first group – not very numerous – comprises cancer stem cells (CSCs), i.e. cells with a low differentiation level, high self-renewing potential *in vivo* and high tumorigenic potential [9, 10]. The cancer stem cell concept is accepted for several types of tumors [2, 11]. However, data for human melanoma are conflicting [2, 12].
2. The second group comprises cells which are considerably more numerous, more differentiated, with only low potential of self-renewal. These cells are non-stem cells of cancer, which constitute the main basis for the tumor bulk [13].

Cancer stem cells are defined as cells that can induce *de novo* tumor formation, self-renew *in vivo* and reestablish the cellular composition of the parental tumor [14]. The CSCs, like their stem cell counterparts in normal tissue, are characterized

by a low level of differentiation and a high self-renewing potential [15]. By asymmetric cell division CSCs develop either into the next generation of CSCs or a more differentiated progeny which has lost the tumor-initiating potential of the CSCs and constitute the second above-mentioned group. Moreover, CSCs have a high tumorigenic potential and a high resistance to apoptosis. These features make them highly responsible for such cancer features as invasiveness, proliferation potential, therapy resistance and ability to recur [8, 9].

As mentioned above, different data concerning cancer stem cells for human melanoma are contradictory [16, 17]. It may be a result of applying an inappropriate protocol for the isolation of cells with such properties. Isolation of such cells requires a very gentle method of cell fractionation, with omission of the stage of trypsin incubation. In other cases, strong proteolytic activity may selectively damage markers of stemness [2, 3, 18] and, in turn, isolated cells might lose their hypothetical properties of stem cells.

As proved by Civenni *et al.*, a special protocol for the isolation of such cells, in the absence of trypsin activity, allowed CD-271-positive melanoma cells to be obtained, and these were able to give rise to primary tumors in all cases in nude and NOD/SCID mice. Moreover, xenografts were the exact phenocopies of the parental tumors with respect to the presence of all stem cell markers tested (e.g. CD-271) [2]. The full heterogeneity of human melanoma on xenotransplantation into nude and NOD/SCID mice was generated, which suggests that CD-271 positive cells are multipotent.

A study by Civenni *et al.* also revealed that CD-271-positive cells after re-transplantation from the primary tumor bulk were able to generate secondary tumors in nude mice. Moreover, CD-271-positive cells re-isolated from secondary xenografts generated tertiary tumors.

In contrast, CD-271-negative cells were unable to generate secondary or tertiary tumors after re-transplantation from the primary tumor bulk. These results allowed the authors of this experiment to conclude that CD-271/SOX10-positive cells might be treated as melanoma stem cells, which, by definition, can induce *de novo* tumor formation [2].

Even those few representatives of CSCs, due to the high self-renewing potential, will rebuild the full structure of a tumor. Hence, planning targeted therapy focusing only on destroying the wider group (non-stem) of cells and leaving even a small amount of CSCs may be treated as an instance of professional error. This kind of therapy would be correlated with temporary remission and rapid recurrence as a result of CSC proliferation. In order to be effective, anti-melanoma therapies should involve destruction of both MSC and melanoma non-stem cells [3, 18]. That implies the necessity of using a two-component drug, aimed at destroying both types of melanoma cells. Since the SOX10-MITF pathway, as described below, turned out to be indispensable to the proliferation and propagation of melanocytes with markers of stem cells, gene-targeted therapy which blocks the SOX10-MITF pathway could be considered as the first component. The second component should destroy melanoma non-stem cells, which constitute the mass of the main tumor. Here, patients may be administered drugs of standard chemotherapy (e.g. paclitaxel), immunotherapy (e.g. PD-1 inhibitors) or radiation therapy [19].

SOX10 gene: protein

The gene for SOX10 is among genes whose activity is pivotal for both melanocyte stem cells and the melanocytes after malignant transformation (more details are provided in Table II). The gene encodes the SOX10 protein, which acts as a transcription factor. As a regulator of the SOX10-MITF pathway it is involved in the differentiation of pigment cells during embryogenesis (Figure 1).

Table II. Characteristics of SOX10 functions in melanocytes vs. melanoma cells

SOX10 function during physiological development	SOX10 function during melanoma development
SOX10 is essential in survival of NCCs [68]	SOX10 is required for maintenance of proliferative melanoma cells [6]
SOX10 directly regulates expression of MITF [69]	SOX10 increases the number of CD-271-positive tumor-initiating cells [6]
SOX10 influences melanocyte differentiation [69]	SOX10 via RB protein is involved in cell cycle regulation [29]
SOX10 regulates the dopachrome tautomerase gene, which is involved in melanin biosynthesis [70]	SOX10 regulates migration of melanoma cells by upregulating expression of MIA [29]
SOX10 regulates proper development of the entire nervous system [71]	SOX10 protects melanoma cells from senescence [28]
SOX10 is important in glial fate determination [34]	SOX10 has a pivotal role in nestin expression [5]

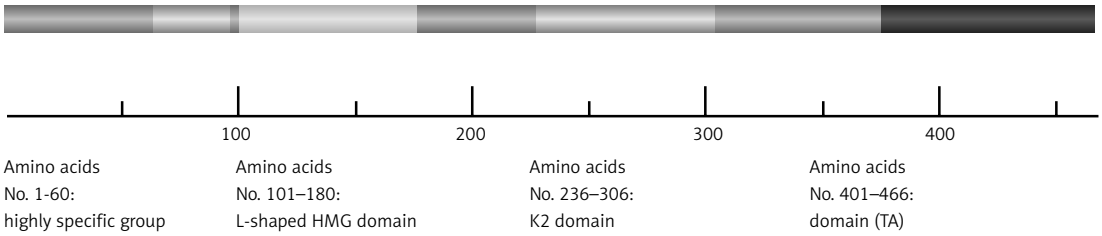

Figure 1. Canonical isoform of SOX10. It comprises 466 amino acids. Characteristic structural components of SOX10 are: DNA-binding HMG domain, transactivation domain K2 and main transactivation domain TA

SOX10-MITF pathway activity lasts during all stages of differentiation of neural crest stem cells (NCSCs) to melanocytes [20, 21]. Then, it decreases and is replaced by the expression of SOX9 [6, 7, 9]. However, its expression is re-induced during tissue repair and also malignancy [22].

Influence of SOX10 on CD-271 and CD-133 surface markers

Recent studies have revealed that knocking down SOX10 leads to changes in melanoma gene expression; e.g. it was proved that the downregulation of SOX10 resulted in a simultaneous reduction of MITF and increased SOX9 expression [6]. Furthermore, a study by Shakhova *et al.* showed that SOX10 downregulation may lead to weaker CD-271 expression [6]. Taking into account the roles of these molecules in melanoma cells, these changes would bring about a strong reduction of proliferation. It could also explain why SOX10-melanoma cells injected into a mouse model system are unable to induce growth in mice [2, 6, 8, 23].

Stem cells are characterized by a few phenotypes which occur consecutively. A study by Redmer *et al.* revealed that CD-271 expression is a necessary step during MSC conversion to the CD-133-expressing phenotype [8]. During this conversion CD-271$^+$/CD-133$^-$ cells become CD-133$^+$/CD-271$^-$. Presumably, the mechanism which is responsible for the loss of CD-271 phenotype involves the occurrence of transforming growth factor β (TGF-β). The latter is a repressor of SOX10 and it also modifies CD-133 expression during NCSC development. The TGF-β occurrence strongly reduces SOX10 expression during NCSC development.

Likewise, when TGF-β appears in MSCs, the SOX10 level decreases, CD-271$^+$/CD-133$^-$ phenotype disappears and conversion to CD-133$^+$/CD-271$^-$ occurs. Here, the same molecular mechanism appears to regulate SOX10 expression during NCSC development and during melanoma progression [2, 8, 24–26]. Since CD-133 accounts for the tumorigenic potential of MSCs, which also increases the potential of metastasis formation, its inhibition may be of therapeutic significance [27].

SOX10 influence on the biology of melanoma

To provide conclusive evidence that SOX10 influences melanoma cell biochemistry, researchers conducted *in vitro* experiments in melanoma cell lines involving the downregulation of SOX10 by using either shSOX10 or siSOX10. Findings of independent studies conducted *in vitro* by Cronin *et al.* [28] and Graf *et al.* [29] revealed that after shSOX10 or siSOX10 administration melanoma cells became enlarged, flat and translucent and showed slower or completely arrested cell growth at the G1 phase. Furthermore, 72 h after transfection the cell death rate significantly increased.

The explanation of *in vitro* cell cycle arrest is the decreased level of RB protein, which is the crucial protein in cell cycle progression. Apparently, SOX10, as shown in Figure 2, is responsible for regulating RB1 expression [28]. Additionally, Shakhova's *et al.* RT-PCR experiment showed that after downregulating SOX10 protein in human melanoma cells, there was a huge decline in expression of the antiapoptotic factor Bcl-2 as well as a simultaneous rise in expression of the proapoptotic factor caspase-1 (CASP1). Since CASP1, a cysteine protease, could initiate or execute cellular programs leading to cell death, its occurrence in SOX10 sh-RNA expressing melanoma cells may present a reasonable explanation of the influence of SOX10 on melanoma cell vitality [6].

Cells after immunostaining for β-galactosidase (SA β-gal), a marker whose level has been correlated with cell senescence, showed increased β-galactosidase activity in SOX10 sh-RNA expressing melanoma cells. Immunostaining with chromatin binding protein (HP1β), for senescence-associated heterochromatin foci (SAHF), another marker of senescence, showed that chromatin of the cells treated with shSOX10 was significantly rearranged, resulting in a larger stained area as compared with the control lines (stably transduced with a non-silencing control hairpin). These results support the hypothesis that the melanoma cell undergoes senescence when SOX10 is knocked down [28, 29].

Another study also revealed that SOX10 inactivation leads to SOX9 upregulation [7]. These two factors seem to be inversely correlated and give

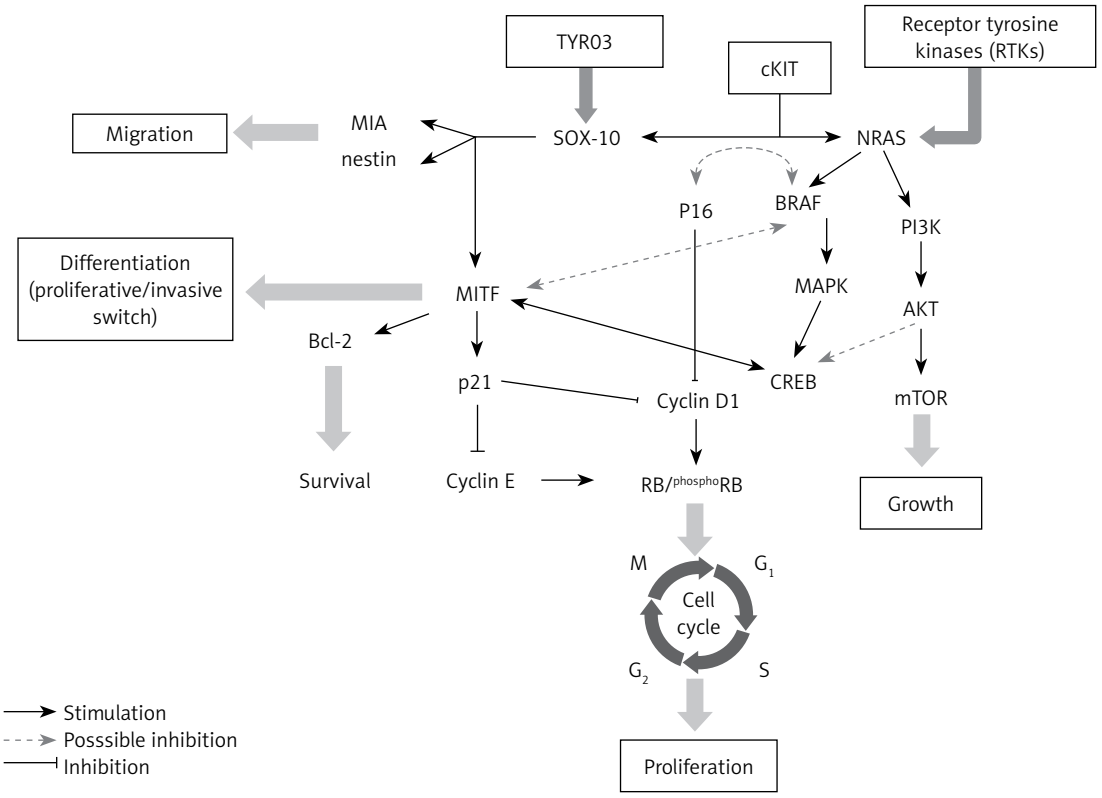

Figure 2. Signal transduction in melanoma with regard to the role of the SOX10-MITF pathway. Apart from direct contribution to melanomagenesis, the SOX10-MITF pathway could also act indirectly by mediating between oncogenes and the cell transcription machinery. Furthermore, in an already developed tumor SOX10 is responsible for its survival and decides about its biochemistry, regulates the cell cycle and influences spreading potential

opposite effects. While SOX10 promotes melanoma formation and acts as an inhibitor of apoptosis in melanoma cells, increased SOX9 level – by arresting the cell cycle – induces proapoptotic processes [7]. Consistently, a study by Verfaillie *et al.* confirmed the SOX10-MITF pathway to be a master regulator of the proliferative gene network [30].

Overall, the above-mentioned studies confirmed the significance of SOX10 for melanoma cell survival and proliferation and also proved the senescence induction when SOX10 is silenced. Since senescence itself appears to be a powerful tool for suppressing tumor cells, si/shSOX10 ability to trigger it has great therapeutic significance [6, 28, 29]. However, the interpretation of the results of the above-mentioned studies requires great caution, as the results of *in vitro* experiments do not necessarily correspond to the *in vivo* ones. It is considered that some melanoma cells – depending on a given context – may grow without SOX10 or MITF.

SOX10 participation in melanoma invasiveness

The ability to migrate, characteristic for neural crest cells (NCCs), appears similar to the spreading ability (metastasizing) typical for melanoma cells. Considering that melanoma cells develop from melanocytes and melanocytes develop from the derivatives of NCSCs during embryonic development, it should be taken into account that the migration of neural crest cells and melanoma cells is potentially regulated by the same mechanism.

Indeed, numerous facts indicate that the spread of melanoma cells and migration of NCSC derivatives are regulated by expression of a similar set of genes [2, 4, 8, 27, 28, 31–35].

To confirm whether SOX10 belongs to the group of genes engaged not only in NCC migration but also in melanoma cell spreading, *in vitro* and *in vivo* invasion assays were performed. *In vitro* evaluation involved the use of Matrigel-coated pored inserts. The aim of using the Matrigel assay was to determine whether the SOX10-inhibited melanoma cells (inhibited by transfection with siSOX10) would invade toward the fibroblast medium. The result was a considerable reduction in the invasion capacity of SOX10-inhibited melanoma cell lines compared to the control (non-inhibited) group [29].

The *in vivo* experiment involved using an embryonic chick model [29]. The experimental group (comprising SOX10-inhibited melanoma cells) and control group (consisting of cells treated with

control siRNA) were injected into the rhombencephalic brain vesicle of developing chick embryos. The effect was assessed by immunostaining chick embryo sections. An essential reduction in the invasion into surrounding mesenchymal host tissue was revealed in the experimental group in contrast to a high level of invasion in the case of the control group. It shows that SOX10 has a critical role in melanoma cell invasion [29].

The same study proved that the factor which links SOX10 with melanoma cell invasiveness is melanoma inhibitory activity (MIA). The MIA is secreted in migrating melanoma cells. It cooperates with cell adhesion receptors and extracellular matrix molecules, promoting cell invasion and migration. Therefore, it is decisive for the spreading potential. As it turned out, SOX10 binds directly to the MIA promoter. Thus, the silencing of SOX10 in melanoma cell lines results in strongly reduced expression of MIA. Consequently, the spreading potential decreases [29, 36].

SOX10 seems to play a role in melanoma spread also through its influence on nestin expression. Nestin is an intermediate filament protein which is involved in cell shape remodeling [37–40]. It is also a neural progenitor marker which is constitutively expressed by neural crest related progenitor cells [41]. Tanaka *et al.* stated that SOX10 activates nestin enhancer, and thus upregulates nestin expression [42]. Brychtova *et al.* stated that nestin expressing cells accumulate peripherally in the tumor. These areas are composed of malignant cells with a higher invasive potential. Nestin is believed to influence the infiltrative growth of malignant cells by facilitating higher cellular dynamics, thus triggering melanoma metastasis formation [43, 44]. Consequently, downregulating SOX10 in melanoma cells would reduce nestin expression and decrease the spreading potential [5].

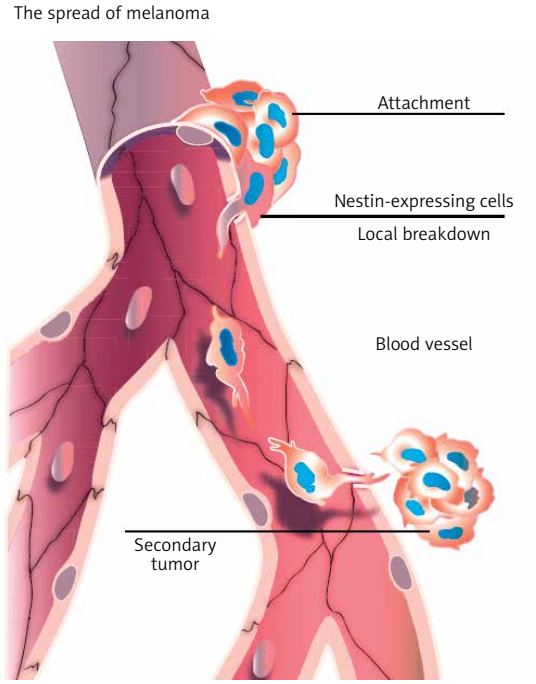

Figure 3. Nestin-expressing cells accumulate peripherally in the tumor. These areas are composed of malignant cells with a higher invasive potential. Nestin is believed to influence the infiltrative growth of malignant cells by facilitating higher cellular dynamics, thus triggering melanoma metastasis formation. Nestin-expressing cells acquire migratory properties allowing them to enter the bloodstream and spread

In another study, Civenni *et al.* found that the number of CD-271/SOX10-positive cells was increased in metastatic melanoma, whereas biopsies of primary, mostly non-metastatic, melanoma did not contain CD-271/SOX10-positive cells. Here, both CD-271 and SOX10 were shown to be factors determining tumor aggressiveness [2].

However, a recent study by Verfaillie *et al.* [30] showed that samples of the 'invasive' type of melanoma express low levels of MITF, whereas the expression of epithelial-to-mesenchymal transition (EMT)-related transcription factor ZEB1 and genes involved in TGF-β signaling is high. Since SOX10 is an MITF pathway regulator, this study seems to be consistent with the above-mentioned studies by John *et al.* [26] and Redmer *et al.* [8], which proved TGF-β to epigenetically modify the expression of CD-133 and reduce the level of SOX10.

Samples of 'proliferative' type of melanoma have high levels of MITF. It may be possible that melanoma invasiveness is triggered by the appearance of clusters of MITF-low/ZEB1-high cells at the edge of the primary lesions [30]. These cells acquire migratory properties allowing them to invade the dermis and enter the bloodstream. These properties could be strengthened when nestin is expressed simultaneously. This discrepancy may be a result of melanoma cell ability to switch back and forth between these transcriptional states, as was confirmed when SOX10/MITF-positive cells were also found at metastatic sites [30]. The way that cancer forms metastasis is shown in Figure 3.

Mutations leading to melanoma. Signal transduction in melanoma with regard to the role of SOX10

Since only one mutation would lead to melanocyte senescence rather than to malignant transformation into a melanoma cell, melanoma is said to be an effect of accumulation of oncogenic mutations. To date, there have been a few known mutations which contribute to melanomagenesis. Some of them are 'driving mutations'. Below, there is a description of mutations which are very important in melanomagenesis (Figure 2).

BRAF gene mutation

An oncogenic BRAF mutation is a very common activating mutation in melanocytes. BRAF is a gene that makes the B-Raf protein, involved in directing cell growth. In some cancers, e.g. in some melanomas, it is mutated and causes activation of the mitogen-activated protein kinase (MAPK) signaling pathway. As a result, the activating signal is transduced to the nucleus and then the cell starts its uncontrolled growth and proliferation. According to the literature it occurs in over 50% of melanomas. Cronin found BRAF-activating mutations in 76% of metastatic tumor samples [33]. Moreover, as it turned out among 11 tissues of melanoma metastasis harboring mutations in the SOX10-MITF pathway (previously confirmed), 10 also had activating mutations in BRAF.

Nevertheless, it remains unclear whether those additional mutations in the SOX10-MITF pathway in combination with BRAF mutation have an antagonistic or synergistic effect for melanomagenesis [33]. A study conducted by Wellbrock and Marais showed that MITF gene expression was suppressed by oncogenic BRAF. Forced MITF re-expression counteracted the proliferation of melanocytes with BRAF oncogene mutations. Hence, the SOX10-MITF pathway seems to act as a suppressor in BRAF-activated tumors [45].

KIT gene mutation

Expression of the KIT cell receptor was found to be upregulated in cutaneous melanoma [31]. KIT is the receptor tyrosine kinase (RTK) for stem cell factor (SCF), a crucial mitogen and survival factor for melanocytes and melanoblasts [46]. Activating mutations in the KIT gene are rare. However, when they occur, their oncogenic potential is said to play an important role in melanoma development [6, 47–50]. A study by Ronnstrand showed that upregulated KIT expression is correlated with increased SOX10 expression [50].

TYRO3 (receptor protein tyrosine kinase) gene overexpression

Overexpression of TYRO3 influences SOX10 nuclear localization, which is raised, and also leads to increased MITF-M expression. Therefore, oncogenic pathways are activated by upregulated expression of both RTK KIT mitogens (responsible for the development and survival of the cells) and Bcl-2 antiapoptotic protein. In the end, activation of the mitogen-activated protein kinase (MAPK) pathway leads to increased expression of CREB transcription factor, which multiplies MITF expression. Here, positive feedback occurs. As a result, a cell which is already malignant avoids senescence and starts proliferating [49–56].

SOX10 and MITF gene mutations

SOX10 gene mutations

Since SOX10 is an MITF pathway regulator and there is also direct cross-activation between MITF and SOX10 [30] the significance of SOX10 mutations is high [57–59]. It may be deduced that although maintaining a wild-type SOX10 gene version is more profitable for melanoma formation and survival, there are few SOX10 mutations which could upregulate the SOX10-MITF pathway and hence, in some cases, facilitate melanomagenesis [28, 33, 55]. A study by Cronin *et al.* demonstrated prevalence of SOX10 mutations in primary melanomas (6/55 samples) as well as in metastatic melanomas (3/50 samples) [33]. In total, 9/105 (8.6%) analyzed samples harbored SOX10 mutations.

MITF gene mutation

MITF amplification, overexpression and MITF single base substitutions are common in melanomas [60]. These mutations could also contribute to melanomagenesis [6, 33, 54]. This is a result of removing MITF control of the cell cycle by changes in MITF transcriptional activity or MITF stability. The most obvious explanation is losing ability to activate the p21 promoter [61]. Then, due to the lack of p21, melanoma cells might escape from p21-dependent cell cycle arrest and start uncontrolled proliferation [4, 28, 33, 49, 53, 62].

Cronin demonstrated the prevalence of MITF mutations in primary melanomas in 2/26 samples. In melanoma metastases MITF was somatically altered in 8/50 (16%) [33]. A similar rate of changes was reported in the case of MITF locus amplification. It was 10% for primary cutaneous aberration and 20% for metastasis [60, 63–65] (Table III).

NRAS gene mutation

NRAS oncogene makes NRAS protein which, when constantly active, leads to tumor growth and survival. NRAS mutation affects MAP kinase (MAPK), and it is often observed in metastatic melanoma. NRAS is said to be mutated in 25% of melanoma cases. Cronin *et al.* observed oncogenic NRAS mutations in 12% of metastatic tumors (6/50) [33].

Moreover, Shakhova's *et al.* research showed high expression of the NRAS oncogene to be associated with an increase in SOX10 expression [6, 7]. During this research the investigators found that haploinsufficiency for SOX10 (*Tyr::Nras*^{Q61K}*INKa*$^{-/-}$ SOX10$^{Lac/+}$ mice) prevents proliferation of cells

with overexpression of the NRAS oncogene and could provide an effective way to stop melanomagenesis in mice which, by presence of the NRAS mutation, are genetically predestined to develop this tumor [6, 7].

INK4a mutation

Cells could also be affected, even simultaneously, by the reverse kind of genetic defect, namely the lack of a tumor suppressor. In the case of melanocytes the suppressor is $p16^{INK4a}$. INK4a-deficient melanocytes (cells with inactivated tumor suppressor) lose their anti-cancer protective mechanism, and without that shield these cells are susceptible to malignant transformation [13]. Mutations in the INK4a locus are the most common in human melanoma. As Shakhova's *et al.* research documented, SOX10 haploinsufficiency protected INK4a-deficient mice injected with melanoma cells from developing melanoma [6].

Role of SOX10 in melanoma therapy resistance

Previous years introduced the most important anti-melanoma drug – vemurafenib. It is an example of targeted therapy against mutated BRAF protein, as vemurafenib is useful only in the case of this particular mutation. The response rate is very high, but development of therapy resistance is becoming problematic.

As it turned out, the reason for the resistance is the triggered expression of epidermal growth factor receptor (EGFR) as a response of cancer cells during drug selection [66]. Although EGFR is not a common protein in melanoma, in the presence of vemurafenib the fact that some cells possess it gives them drug resistance, which is an obvious advantage over cells without EGFR expression. The cells without EGFR are decimated by vemurafenib. This small set of $EGFR^+$ cells could fill the space resulting from the $EGFR^-$ cells killed by the activity of vemurafenib. Thus, vemurafenib plays the role of a selective factor which enhances the acquisition of EGFR expression by melanoma cells, which would normally be disadvantageous for the BRAF-mutated melanoma cell (as it would reduce the potential of proliferation) [67].

As recently published studies show, the factor which is crucial for acquiring higher EGFR expression is SOX10. Suppression of the SOX10 gene induced higher expression of EGFR. Although silenced SOX10 would be very unprofitable for melanoma in the absence of vemurafenib, when this compound is present the cells with silenced SOX10 are protected and thus resistant to the therapy. Then, it is the factor which allows some melanoma cells to survive [67]. Hence, gene therapy against SOX10 and combined therapy with vemurafenib seem to be mutually exclusive.

Conclusions

Melanoma is one of the most complicated neoplasms in terms of the number of mutations occurring at the same time. The rapid growth of human gene therapy is probably the greatest chance for oncology to develop a new treatment with a potential to target melanoma cells specifically while sparing normal tissues. To take advantage of this potential appropriately it is necessary to direct it towards the pathway which is possibly the most crucial for the development of melanoma. This article describes the SOX10-MITF pathway, which seems to meet this criterion.

For more than 10 years we have collected more and more evidence that these two proteins play an important role in melanomagenesis (Table IV). The SOX10-MITF pathway is a well-documented conduit between oncogene, cell transcription machinery and melanomagenesis.

Animal experiments revealed that the most common mutations responsible for melanoma

Table III. Characteristics of MITF functions in melanocytes vs. melanoma cells

MITF function during physiological development	MITF function during melanoma development
MITF controls the cell cycle and thus prevents uncontrolled proliferation [64]	Elevated expression counteracts BRAF-stimulated melanoma cell proliferation [45]
MITF is critically required for survival of melanoblasts [72]	MITF is clearly required for melanoma proliferation and progression [53, 64]
MITF regulates melanocyte migration [54]	MITF regulates melanoma cell shape and invasiveness [74]
MITF is involved in differentiation of melanocytes [73]	Reduced MITF stability and/or transcriptional activity leads to removing MITF's tight control of the cell cycle [33]
MITF is crucially important for survival of postnatal melanocyte stem cells [15]	MITF functions as a 'lineage addiction' oncogene in malignant melanoma [55]

Table IV. Characteristics of stated SOX10 features in melanoma. The table shows authors who examined a particular SOX10 function

Characteristic feature	Bakos [4] (2009)	Flammiger [5] (2009)	Cronin [33] (2009)	Cronin [28] (2013)	Shakhova [6] (2012)	Graf [29] (2014)	Redmer [8] (2014)	Shakhova [7] (2015)
SOX10 presence	√ 45%	√ 75%	√ 90%	√ 66%	√ 90%	√ 66%	√	√
siSOX10 arrested cell cycle				√	√	√		√
siSOX10 induced cell death				√	√	√	√	√
siSOX10 reduced invasion capacity						√	√	
siSOX10 reduced nestin expression	√	√				√		
siSOX10 reduced number of CD-271$^+$ cells					√		√	
SOX10 haploinsufficiency led to senescence				√	√			√

formation seem to be less dangerous after knocking down the SOX10-MITF pathway. For example, studies involving siSOX10 showed that silencing SOX10 both in human melanoma cells and in a genetic melanoma mouse model results in decreasing cell vitality, arresting the cell cycle, and inducing senescence and apoptosis [6, 28, 29]. Through its influence on MIA and nestin expression SOX10 facilitates metastasis formation [29, 36, 43].

Furthermore, the idea of cancer stem cells, first described by Nishimura *et al.*, seems to correspond with melanomagenesis [15]. Numerous recent publications on melanoma report the occurrence of cells with melanocyte stem cell phenotype during melanoma formation. The characteristic feature of them is the presence of multipotent cell markers, e.g. CD-271$^+$. Cells with that phenotype seem to have melanoma-initiating cell features, and SOX10 seems to be required for development of the CD-271$^+$ phenotype in transformed melanocytes [2]. If this idea is correct, stem cell markers in harmony with the SOX10-MITF pathway will determine their tumorigenicity, heterogeneity, plasticity and stem-like properties. After division these cells will develop into new tumor-initiating cells or a more differentiated progeny without stem-like properties but instead will constitute tumor bulk [2, 6, 8].

Although melanoma stem cells still remain a hypothesis requiring further evaluation, data reviewed in this article suggest that in the future therapeutic silencing of the SOX10-MITF pathway can be developed as a drug used in combination therapy against melanoma.

Conflict of interest

The authors declare no conflict of interest.

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