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Insights of Neuro-Oncology

Original Article Open Access

Sox2 Functions in Neural Cancer Stem Cells: The Importance of the Context

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Abstract

The Sox2 transcription factor is expressed in different neural tumors. In particular, it is active within the "cancer stem cell" (CSC) subpopulation of tumor cells, able to reinitiate tumorigenesis after conventional chemotherapy (to which it is usually resistant). This led to hypothesize that Sox2 (and its downstream regulated genes) may qualify as promising targets for therapeutic strategies directed against CSC. However, the potential relevance of Sox2 in this regard depends on whether it is functionally important to maintain CSC. Here, we comparatively examine the effects of Sox2 genetic ablation within mouse models of different neural tumor types. Sox2 ablation in mouse glioma (and in human glioblastoma-derived CSC) demonstrated a critical function for Sox2 in the maintenance of CSC. Surprisingly, however, Sox2 ablation in two different mouse models of melanoma (a neural crest-related tumor), and in a mouse model of medulloblastoma of the Sonic Hedgehog subgroup, showed that, in these contexts, Sox2 is dispensable for tumorigenesis. This heterogeneous situation has a parallel in the normal development of the nervous system, where generalized Sox2 ablation in neural stem/progenitor cells selectively affects the development of some neural regions, but not other ones. Molecular mechanisms underlying these specificities may involve the regulation, by Sox2, of different sets of target genes in different tumors, but also a redundant regulation of the same targets by different Sox transcription factors, differentially coexpressed with Sox2 in different tumors. Collectively, these findings point to the need to experimentally address the requirement for Sox2, and its downstream targets, within different tumor types, as a prerequisite to fully exploit its potential as a target for novel therapeutic approaches.

Keywords

Sox2, Cancer stem cells, Cancer, Glioblastoma, Oligodendroglioma, Medulloblastoma, Melanoma, Mouse genetic models

Abbreviations

CSC: Cancer Stem Cells; ESFT: Ewing Sarcoma Family Tumors; MB: Medulloblastoma; NSC: Neural Stem Cells; SCC: Squamous Cell Carcinoma; SCLC: Small Cell Lung Carcinoma

Introduction

Stem cells are "cells that have the ability to perpetuate themselves through self-renewal and to generate mature cells of a particular tissue through differentiation" [1]. "Cancer stem cells" (CSC) were identified in human brain tumors [1-3], as a minority subpopulation able to reinitiate tumor development following conventional chemotherapy (to which they are usually resistant), or, experimentally, following transplantation in a host (mouse) brain. Much evidence has accumulated supporting the importance of CSC for tumor relapse and propagation [2,4,5], and CSC are now considered a central target for therapeutic approaches aimed at eradicating tumor development. CSC self-renew, and also produce "pseudo-differentiated" cells, constituting the tumor bulk [6-8]. While most of the tumor bulk cells are typically proliferating (though they are called "differentiated" from the CSC perspective), CSC can be slowly proliferating and even "quiescent" [6,8], making the use

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Accepted: October 13, 2018;

Published online: October 15, 2018

Citation: Barone C, Pagin M, Serra L, et al. (2018) Sox2 Functions in Neural Cancer Stem Cells: The Importance of the Context. Insights Neurooncol 2(1):18-26

of drugs hitting proliferating cells uneffective in killing them.

Sox2 is a member of the *S*ry-related HMG-b*ox* (SOX) family of transcription factors; the Sox2 gene is located on chromosome 3, in both humans and mice, and in both species it is constituted by a single coding exon [9]. Sox2 was first studied in the context of normal embryonic development, where knock-out experiments in mouse demonstrated its essential role to maintain the pluripotent stem cells of the early embryo [10], as well as its function in several tissue-specific stem cells, including neural stem cells (NSC); NSC cultured *in vitro* from the mutant neonatal mouse brain fail to self-renew in long-term culture, and, *in vivo*, postnatal hippocampal NSC are impaired [11-14].

The discovery of CSC in tumors focused attention on Sox2 also from the point of view of this pathological stem cell type. Indeed, Sox2 is expressed in different tumors of neural origin, such as gliomas (the most common primary brain tumors, whose most malignant and lethal subtype is glioblastoma multiforme), medulloblastomas (the most common brain tumor in childhood), and melanoma (a tumor arising from neural crest type cells); In all of these tumors, CSC have been identified, and found to express Sox2 [2,8,15].

In this paper, we focus on the comparative review of recent experiments, that made use of conditional mutation of Sox2 in different mouse models of neural tumors (and of genetic Sox2 ablation in human CSC-enriched cultures of the same tumor types), to investigate the functional relevance of Sox2 in tumorigenesis, and, in particular, in the maintenance of CSC. These experiments demonstrated an absolute requirement for Sox2 of CSC able to reinitiate tumorigenesis of gliomas (glioblastoma and oligodendroglioma) in mouse; unexpectedly, however, they also showed that Sox2 is dispensable for tumorigenesis in a model of Sonic Hedgehog (SHH)-subgroup medulloblastoma, and in two different models of melanoma. We discuss a parallel of this situation with the identification of region-specific Sox2 functions in the context of the development of the normal neuroepithelium, and possible molecular mechanisms underlying the context-specificity of Sox2 functions. Finally, we discuss the need and approaches to identify functional downstream effectors of Sox2 in Sox2-dependent CSC, that could complement Sox2 as targets of therapeutic strategies directed against CSC.

Sox2-dependent neural cancer stem cells in gliomas

Gliomas are the most common cerebral neoplasias (86%) [16-18]. Glioblastoma multiforme (GBM), the most aggressive and deadly among gliomas (average patients' survival is about 15 months), was one of the first tumor types in which CSC were originally described (reviewed in [2]. The development of serum-free *in vitro* cultures of tumor-derived cells has allowed to expand CSC (in equilibrium with more differentiated progeny) from GBM tissue of many different patients; these cells retain the ability to re-form a tumor with the same characteristics of the tumor of origin following transplantation into a host mouse brain (xenograft), and thus represent important *in vitro* models of CSC. GBM, and CSC-enriched cultures derived from it, consistently express Sox2 [19].

Gangemi, et al. [20] first addressed the consequences of lowering Sox2 levels in some patient-derived CSC-enriched cell cultures, by expressing anti-Sox2 shRNAs causing strong reduction of Sox2 mRNA levels. This resulted in reduced cell proliferation and reduced clonogenicity *in vitro*, and to loss of tumorigenicity *in vivo*, in mouse xenografts [20]. A role for Sox2 in maintaining other patient-specific GBM CSC was further supported in additional important work by other laboratories [15].

In mouse cells, complete Sox2 ablation was obtained *in vitro* by conditional knockout in a genetically defined model of glioma: a high-grade oligodendroglioma induced by overexpression of Platelet-derived growth factor B (PDGF-B) [21]. Oligodendroglioma is the second most common tumor in adults; patients with high-grade oligodendroglioma have a median survival of 3-4 years [16,22]. Alterations of PDGF-B signalling are common molecular lesions in human gliomas, including oligodendrogliomas: PDGF and PDGF receptor have both been found overexpressed in glial tumor-derived cells and glioma surgical samples, and amplification of the gene encoding the PDGF receptor-A occurs in high-grade oligodendrogliomas [23-25]. In addition, PDGF can initiate the "reprogramming" of normal, committed O2A oligodendrocyte progenitors to neural stem-like cells, in a process that requires Sox2 [26]. PDGF-B was used to induce tumor development in embryonic brain; cells cultured from such tumors would re-initiate tumor development following transplantation, thus behaving as CSC. Sox2 ablation was achieved by conditional knockout, in which Cre recombinases delete the endogenous Sox2 gene, that has been previously flanked by loxP sites, the Cre substrate ("Sox2 flox" allele) [13]. Sox2 Cre-mediated ablation *in vitro* in such glioma cells completely prevented tumor reinitiation following *in vivo* transplantation; over the time window in which non-deleted cells developed deadly tumors, mice transplanted with Sox2-deleted cells remained tumor free. The only two tumors developing from Cre-treated cells were SOX2-positive, demonstrating they were derived from non-deleted cells. *In vitro*, Sox2 ablation caused proliferation reduction, apoptosis activation, and aberrant

differentiation into cells expressing oligodendrocyte and astrocyte markers [21]. Counterintuitively, Sox2, or peptide fragments of it, though being a nuclear protein, can be found exposed on the cell surface of tumor cells, in association with the Major Histocompatibility Complex (MHC), presumably as a result of tumor cell lysis and processing of its proteins; based on this, immunotherapy against SOX2 protein was attempted, which resulted in a doubling of survival time of mice transplanted with oligodendroglioma cells [21].

Sox2 cooperating genes

Recent experiments investigated transcription factors cooperating with Sox2 in cell "reprogramming" to tumor-propagating cells (cancer stem cells). In recent work [7], SOX2 was able to reprogram "differentiated" GBM cells from human tumors (DCGs) to stem-like tumor-propagating cells (TPC), when transduced into the DCGs together with transcription factors POU3F2 (BRN2), SALL2, and OLIG2. In GBM-derived CSC-enriched cultures grown in serum-free conditions, genomic mapping (by ChIPseq) of H3K27Ac, a histone modification carried by active enhancers and promoters, had initially revealed regulatory elements specific of TPC (versus DCGs), and these were enriched in the DNA sequence recognized by SOX proteins. RNA seq showed that expression of SOX2 (and of SOX1, SOX5, SOX8, SOX21) was higher in TPC than in DCGs ("differentiated" by serum or BMP4 addition). Similarly, transcription factors SALL2, POU3F, and OLIG2 were selected based on two considerations: enrichment, in TPC versus DCGs, of their expression, and the presence of H3K27Ac (an epigenetic mark of transcriptionally active state) on their binding sites on gene promoters and enhancers. The combination of SOX2 (but not SOX1), SALL2, POU3F and OLIG2 (or Rest Corepressor 2, RCOR2, a transcriptional corepressor) could reprogram DCGs to TPCs, that carried a genome-wide pattern of H3K27Ac sites superimposable to that of the TPCs that had been directly grown from the tumor. Further, the 4 factors cobind to a large number of distal regulatory elements specifically active in TPCs. Interestingly, a minority of cells coexpressed the 4 transcription factors in the tumor, as demonstrated by immunofluorescence. Looking for therapeutic implications, two mediators acting downstream to the 4 transcription factors were also identified: RCOR2 (that can replace OLIG2 in the reprogramming cocktail) and histone demethylase LSD1, whose repression caused cell death specifically in TPCs. Interestingly, it had previously been found that LSD1-specific inhibitors impaired the growth of Sox2-expressing, but not that of Sox2-negative, lung squamous cell carcinomas (SCC), and Sox2 expression was associated with sensitivity to LSD1 inhibition in lung, breast, ovarian, and other carcinoma cells [27]. This indicates that LSD1 is a mediator of tumorigenic effects downstream to Sox2, but not other tumorigenic factors.

In a related study, ChIPseq in GBM cells showed that DNAase hypersensitive sites in patient-derived GBM cells, mapped by ATAC-seq (a technique that allows to map DNA regions that are more accessible in chromatin, usually in correlation with the binding of transcription factors), are enriched in DNA recognition sequences for SOX2 and FOXG1, a transcription factor active in embryonic brain development; ectopic expression of SOX2 and FOXG1 in postmitotic astrocytes reactivated proliferation and stem cell properties. The coexpression of FOXG1 with SOX2 in GBM led to hypothesize that they could, together, contribute to cell "reprogramming" to stemness in gliomagenesis [28].

Sox2 relevance for non-neural tumors. Common downstream Sox2 effectors?

Importantly, conditional knockout and RNA interference experiments showed that SOX2 controls CSS functions also in some non-neural tumours, such as in skin, lung, and aesophagus squamous cell carcinomas (SCC), osteosarcomas, Ewing sarcoma, and small cell lung carcinomas (SCLC) [29-33]. This suggests that some downstream effectors of Sox2 function may be conserved between different tumors. In this regard, in osteosarcoma-initiating cells, Sox2 directly represses the genes encoding two activators of the "hippo" signalling pathway, Nf2 (Neurofibromin 2, also called Merlin, encoding a protein involved in connecting the cytoskeleton with proteins of the cell membrane) and WWC1 (also called Kibra, encoding a cytoplasmic phosphoprotein), which in turn negatively regulate the transcriptional coactivator YAP, important for promoting tumor growth; interestingly, Sox2 depletion led to upregulation of Nf2 and WWC1, downregulation of YAP, and to reduced cell clonogenicity, in both osteosarcoma and GBM cells, indicating that these effectors may be shared between these tumor types [30]. In osteosarcomas, as well as in laryngeal cancer cells, Sox2 was also proposed to control migration and invasion via the Wnt/beta-catenin signalling pathway [34,35], though enforced Sox2 expression in lung adenocarcinoma was reported to promote cell migration and invasion, but to inhibit Wnt/beta-catenin signalling activity [36]. Thus, Sox2 might regulate the Wnt/beta-catenin pathway in different tumor types, though not necessarily with the same functional outcome. In hematopoietic tumors, Sox2 expression was detected in cultured cells isolated from ALK-positive anaplastic large cell lymphoma, and Sox2 downregulation impaired their clonogenicity and tumorigenic ability; oxidative stress increased Sox2 expression and cancer

stem cell properties in a subpopulation of cells, and Sox2 was reported to bind DNA more efficiently [37]. It is possible that specific partners had become available in oxygen-stressed cells, or that increased levels of Sox2 were important for binding; alternatively, posttranslational modifications of Sox2 were proposed to be involved in this "activation" following oxidative stress [38].

Interestingly, some papers reported Sox2 expression and some functional effects of Sox2 downregulation also in human mammary cancer cell lines [39,40]. However, in a widely studied mouse model of breast cancer, produced by expression of a transgene encoding a mutated ErbB2/Neu oncogene driven to mammary tissue by the MMTV promoter [41,42], and activated by a mammary-specific Cre recombinase [43], Sox2 ablation had no effect on tumorigenesis (A.B.S. and S.K.N., unpublished observations). It is possible that breast tumors are heterogeneous regarding functional requirements for Sox2 in tumorigenesis. Indeed, Sox2 expression was observed in some breast tumors (mostly belonging to early stages of tumor progression), but not in others, and some, but not all breast tumor-derived stem cell-enriched cultured cell lines are reported to express Sox2 [39]. A future characterization of the gene regulatory networks acting in breast cancer CSC might allow to better categorize them with respect to Sox2 function.

It is interesting to note that Sox2 is important also in the normal cell counterparts of CSC within several of the non-neural tissues (see above) known to develop Sox2-dependent tumors, as previously seen with NSC [13]: indeed, normal osteoblasts, dermal papilla cells, and cells of the developing foregut (giving rise to lung, esophagus and trachaea) require Sox2 function [44-46]. This indicates that Sox2 function, already present in tissue-specific stem/progenitor cells, is retained by CSC of (at least some) tumors of the same tissue type. A different situation was documented in Ewing Sarcoma Family Tumors (ESFT), mesenchimal tumors thought to arise from primary mesenchymal stem cells. Here, SOX2 expression is strongly activated *de novo* by the oncogenic transcription factor that characterizes a high proportion of ESFT, encoded by the EWS-FLI-1 fusion gene. EWS-FLI-1 activates SOX2 (together with OCT4 and NANOG), and SOX2 is a key factor in the emergence of a ESFT CSC population; its downregulation in ESFT cells antagonizes cell proliferation and tumorigenesis [32].

Collectively, these findings show that SOX2 is required by CSC in various gliomas, in mouse and human (as well as by CSC of several non-neural cell types). This raises the possibility that SOX2 may qualify as a target for CSC-directed therapy strategies; note that, though Sox2 is highly expressed in gliomas and CSC, its expression is very limited in normal brain tissue surrounding the tumor. They further indicate that the identification of new downstream mediators of Sox2 function in gliomas may be of relevance for therapy approaches, not only in glioma, but also in other Sox2-dependent tumor types, where some relevant Sox2-controlled gene regulatory networks may be conserved (see also the recent reviews on the subject by [15,47].

Medulloblastoma Development in a Sox2-ablated Mouse Model

Medulloblastoma (MB), the most common brain tumor in childhood, was shown early on to harbour CSC [2,3]. Quite some studies were devoted to the Sonic Hedgehog (SHH) subgroup of MB, representing about 30% of total MB, presenting aberrant SHH signalling because of loss of function of negative regulators (including PTCH1, SUFU), activating mutations of positive transducers (SMO), or amplification of transcriptional effectors, like GLI2 [48]. SHH subgroup MB were shown to originate from cerebellar granule neuron precursors, that proliferate during normal development under the physiological stimulus of SHH [49]. In these tumors, SHH pathway inhibitors entered clinical trials, but reports of resistance and relapse indicate the possibility that an insensitive CSC might be spared [8].

In mouse, a model of the SHH subgroup MB is the irradiated Ptch +/- mouse, where postnatal irradiation increases tumor frequency from 20% to 80%. Recent work in this model has shown that rare, quiescent cells, expressing Sox2, behave as tumor-propagating cells following transplantation, and in primary tumors *in situ* [8]. Sox2-expressing cells, and their progeny, were labelled *in vivo* through activation of a GFP transgene by an inducible Cre (CreERT2) driven by the Sox2 locus, and characterized through tumor development, by immunofluorescence. This revealed that rare Sox2-positive cells (less than 5% of total) produce rapidly proliferating progenitors (marked by doublecortin, DCX), that, together with their non-dividing progeny (positive for NeuN) constitute the tumor bulk. The fraction of Sox2-positive cells increased following anti-mitotic, or anti-SMO, therapy, which kills dividing cells; this suggests that Sox2-positive cells were spared by these therapies and could be responsible for the observed relapse. These findings indicate Sox2-positive cells as a promising target for anti-CSC therapy in SHH subgroup MB but leave open the question of whether Sox2 itself is functionally relevant for them.

Sox2 function in mouse MB tumorigenesis has been tested by conditional Sox2 knockout within another model of SHH subgroup MB, the lox-stop-lox-SmoM2-YFP mouse [50]. SmoM2 encodes a mutated version of the Smo gene, encoding the SHH co-receptor; SmoM2 was

originally discovered as an activating mutation of Smo (ant thus of the SHH pathway) in basal cell carcinomas [51]. In the mouse model, the SmoM2 transgene activates SHH signalling following Cre recombinase-mediated excision of the stop sequence [49]. In hGFAPCre:SmoM-$2^{flox/+}$ mice, SmoM2 is activated throughout the neuroepithelium, and MB develops [49]; these MB expresses Sox2, with sparse cells showing particularly high expression levels [51]. In hGFAPCre:$Sox2^{flox/flox}$:$SmoM2^{flox/+}$ mice, tumors developed, where SOX2 was not detected. However, the development and morphology of the tumors were not significantly altered by Sox2 loss; further, the survival time was not significantly different between Sox2-positive and Sox2-negative genotypes.

It should be noted that, in this model, SHH signalling is activated uniformly within the many cells in which SmoM2 is activated by Cre, which does not mirror the pathological situation in which an oncogenic mutation is first present in just one cell. However, in this model, Sox2 ablation did not preclude MB tumorigenesis. It is possible that, in MB, Sox2 is upstream to genes regulating SHH signalling, as seen in normal NSC [13]; in the SmoM2 model, Sox2 function might be bypassed by autonomous, constitutive activation of the SHH pathway.

In this model, expression of Sox3, a transcription factor belonging to a subgroup of Sox genes coexpressed with Sox2 in the developing neuroepithelium [12], was detected in the tumor, and found upregulated in mutant cerebella following Sox2 ablation [51]. Sox transcription factors co-bind to many target genes [52], so these findings raise the possibility that Sox3 acts redundantly with Sox2 to maintain MB tumorigenesis. Vice versa, pro-differentiative Sox factors (Sox5, 6, 21) were found to be downregulated during malignant glioma progression, their genetic ablation increased the ability of cells to form glioma-like tumors, and expression of high levels of Sox5/6/21 in primary human GBM cells antagonized their tumorigenic capacity [53]. A similar, antagonistic

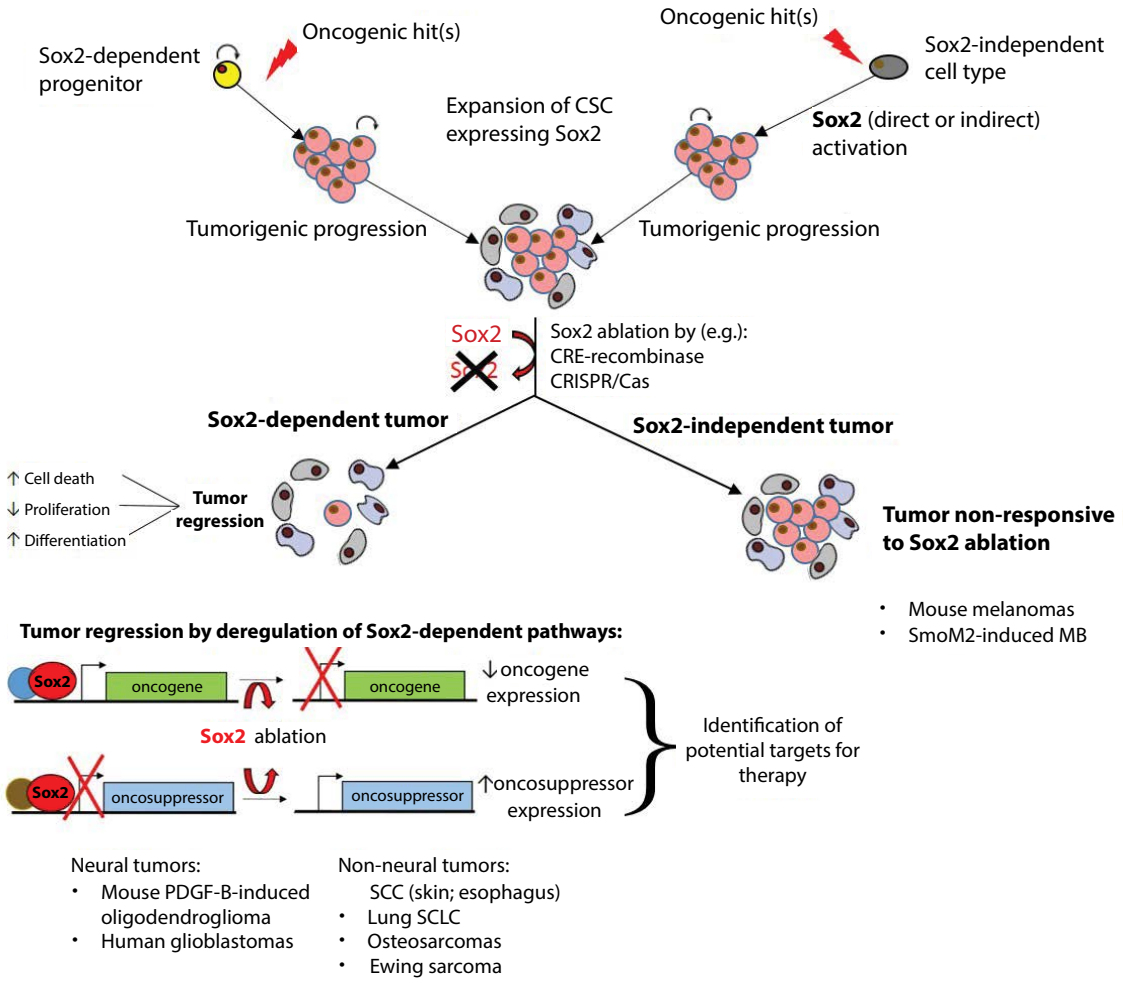

Figure 1: Sox2 experimental ablation distinguishes Sox2-dependent from Sox2-independent CSC.

Oncogenic hits can convert normal cells to CSC. Sox2 ablation can antagonize CSC and tumor progression (left). Here, Sox2 downstream genes can be identified, which vary their expression following Sox2 loss; downregulated as well as upregulated genes are identified. Experimental manipulation of the activity of these targets can identify important mediators of Sox2 effects on cancer cells, providing potential targets for therapy.

function of Sox9 and Sox10 was also described in melanoma [54] (see below Figure 1). It is possible that the Sox genes coexpressed with Sox2 in tumors and CSC may represent a generally important factor conditioning Sox2 function in CSC. Their importance may be tested by genetic ablation experiments, in combination with Sox2.

Sox2-independent Neural Cancer Stem Cells in Melanoma

Melanoma is a skin tumor originating from malignant transformation of melanocytes, cells derived from the embryonic neural crest [55]. About 50% of human melanomas express Sox2 [56]; further, Sox2 has functional roles in normal melanocyte progenitors of the developing neural crest, as Sox2 gradual downregulation permits the differentiation of neural crest (and Schwann cells)-derived melanocyte progenitors into melanocytes [57,58]. Sox2 has thus been considered a candidate to play functional roles also in melanoma. A different Sox factor, Sox 10, had been previously shown by conditional knock-out to play an essential role in melanoma development within a mouse genetic model of $Nras^{Q61K}$-driven melanomagenesis [59], prompting related experiments for Sox2.

The study of Sox2 function in human melanoma-derived cells by RNAi-mediated knockdown approaches had given controversial results. Whereas some Sox2 knockdown experiments indicated a contribution of Sox2 to the maintenance of patient-derived melanoma spheres self-renewal and xenograft tumor development [60], and Sox2 overexpression increased melanoma cell invasion [61], in other reports Sox2 knockdown failed to elicit effects [56]. In more recent experiments, Sox2 was fully ablated via CRISPR/Cas9-mediated mutagenesis in human patient-derived melanoma cells carrying a N-Ras Q61L oncogenic mutation and expressing high Sox2 levels [58]. Following xenotransplantation into immunocompromised mice, Sox2-deleted cells were equally capable of generating tumors than non-deleted cells [58], pointing to a dispensable role for Sox2.

In mouse, Sox2 function in *in vivo* melanomagenesis was recently studied in two different genetic models of melanoma, by conditional Sox2 knockout. The first model is the $Tyr:Cre^{ERT2}$ :: $NRas^{Q61K}$ Ink4a-/- mouse, in which a transgene ($Tyr:Cre^{ERT2}$), expressing the inducible Cre^{ERT2} in melanocytes, is coupled to a Cre-activatable transgene, carrying the oncogenic $NRas^{Q61K}$ mutation, together with a homozygous mutation of Ink4a [58]. This model recapitulates all phases of melanomagenesis from benign nevi formation from melanocytes, to malignant transformation, metastases, and tumor dissemination at distant sites; in this model, the requirement for Sox10 in melanomagenesis had previously been demonstrated [59]. In the second model, a $Braf^{V600E}$ mutation cooperates with Pten loss to induce melanoma [62]. Both tumors express Sox2, at least in a fraction of cells. In both cases, Sox2 ablation was obtained by combining these oncogenic mutations with a homozygous $Sox2^{flox}$ allele; this caused Sox2 deletion, by the same Cre recombinases that activated the oncogenic transgenes. In both cases, Sox2 ablation did not prevent tumor formation, and the kinetics and characteristics of tumor growth did not differ detectably in Sox2-negative and Sox2-positive (control) tumors, nor did the survival; further, to test for Sox2 requirement in different phases of tumorigenesis, deletion was induced before the appearance of primary melanomas, or when metastases formed, and again no difference was observed with controls, despite efficient Sox2 deletion [58,62]. This indicates that, at least in these models, Sox2 is dispensable for tumor development from melanocytes. It is possible that the different findings in some human cell lines and in the two *in vivo* mouse models (see above) reflect a previously unappreciated heterogeneity in melanomas regarding Sox2 requirement. Functional experiments will be important in discriminating, among melanomas, those requiring Sox2 function for tumorigenesis.

A Parallel: Different Requirements for Sox2 by Stem/Progenitor Cells of Different Regions of the Normal Developing Nervous System

It may be interesting to note that Sox2 function is highly context-dependent also in the development of the normal nervous system. Following Sox2 deletion throughout the developing neural tube at embryonic day 11.5 (E11.5) (via a nestin-Cre transgene), development of the hippocampus is severely perturbed, whereas neural development in general is comparatively spared [13]. Following even earlier Sox2 ablation throughout the developing telencephalon, at E9.5 (via a FoxG1Cre transgene), the ventral telencephalon (medial ganglionic eminences, the primordium of the basal ganglia) is essentially lost, as is the olfactory neuroepithelium, whereas the dorsal telencephalon (cortex primordium) is comparatively less affected [63,64]. Sox2 ablation in the developing midbrain/hindbrain led to impaired development of the cerebellar vermis, and of postnatal cerebellar Bergmann glia, but not (or much less) of other cerebellar regions and cell types [65]. These findings indicate that Sox2 normally functions in a stage-, region-, and cell type-specific way in neural cells, driving context-specific gene regulatory networks.

Conclusions and Perspective

We have summarized evidence showing that Sox2 is essential for the maintenance of CSC and tumorigenesis in some neural tumor types, while being dispensable in others.

What molecular mechanisms could be involved in this context-specificity of functions in CSC? It is possible that different stem cell programs control the maintenance of, for example, glioma versus melanoma stem cells, and that only the first one requires Sox2. Alternatively, it is possible that, at least in some cases, Sox2 acts redundantly with other Sox factors, coexpressed with it in some tumor types (e.g. Sox3 in MB, see above). The combined ablation, in model systems, of the Sox genes potentially involved, by conditional knockout or CRISPR-Cas-mediated mutagenesis, should clarify these points.

On the other hand, we noted that a requirement for Sox2 is found not only in neural CSC within gliomas, but also in very different, non-neural tumors, such as skin and esophagus SCC, lung SCLC, and osteosarcomas. Perhaps, although they differ by histology and by cell of origin, these tumors share a "core", Sox2-controlled gene regulatory network, active in their CSC. Thus, it will be important to comparatively characterize the gene regulatory networks controlled by Sox2 in these CSCs.

These findings have implications for therapy approaches. On one hand, they suggest it might be advantageous to "classify" tumours according to the gene regulatory networks that function in the maintenance of their CSC, that in turn might involve shared efficacy of CSC-targeting drugs. On the other hand, they emphasize the need for functional experiments, to address the importance of specific gene products (here, Sox2, and its downstream targets), to distinguish driver from bystander roles, in order to appropriately target future CSC-directed therapy approaches.

Acknowledgements

Work in the Nicolis laboratory has been supported by Associazione Italiana per la Ricerca sul Cancro (AIRC-IG), the European Community (ERANET-NEURON ImprovVision), Telethon, Fondazione Cariplo. We wish to thank Sergio Ottolenghi for insightful discussion.

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