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Review

The dose-dependent effect of SOX9 and its incidence in colorectal cancer

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Abstract A member of the *Sry*-related HMG-box family of transcription factors (SOX9) is a transcription factor that belongs to the superfamily of High Mobility Group (HMG) domain transcription factors. SOX9 is expressed in a variety of tissues, including as the intestinal epithelium, where it is now recognised as an important actor for homeostasis. Beside, a high level of SOX9 has recently been correlated with a good prognosis for stage II colorectal cancers. However, growing evidence indicates that deciphering the function of SOX9 in the intestine has to take into account a dose-dependent effect of SOX9. Given the recurrent controversies and the lack of a state of the art as to whether SOX9 behaves like a tumour suppressor or an oncogen in the intestine epithelium, it is time to provide an update of the accumulated knowledge about the biological function of SOX9 in the intestine and about the role of SOX9 in colorectal cancers.

1. Introduction

As a SOX transcription factor, SOX9 contains a high mobility group (HMG) domain [1], which is a conserved amino-acid sequence that was at first identified in *Sry*, a transcription factor essential for the determination of the male sex [2]. To be considered as a SOX (*Sry*-related HMG box), a protein must contain a HMG domain at

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least 50% homologous with the Sry HMG. To date, 20 Sox proteins have been identified in mice and humans and are classified from subgroup A to H according to the structural homologies over the entire protein. Together with SOX8 and SOX10, SOX9 belongs to the E subgroup. Like all SOX, SOX9 is able to bind an unrestricted consensus sequence, (A/T)(A/T) CAA(A/T)G [3] and to induce a strong DNA bending which brings distal control elements together with proximal transcriptional starting sites within the promoters of target genes [4]. However, growing evidence indicates that, at least in intestine epithelial cells, SOX9 is also able to regulate gene expression regardless its transcriptional activity. For instance, SOX9 is able to regulate protein kinase C (PKC) alpha expression by modulating the activity of Sp1 [5,6].

Several studies clearly demonstrated that a critical dose of SOX9 is essential for life. Indeed, *Sox9* haploinsufficiency causes campomelic dysplasia, a syndrome characterised by severe skeletal malformations and XY male-to-female sex reversal [7], which irrevocably leads to death [8]. Thankfully, this is a very rare situation but this indicates that the available dose of SOX9 is critical for its function and, furthermore, for life. Reduced expression of SOX9 in the embryo has also been shown to result in testis development disorder and sex reversal [9] and, in addition to a critical role in Sertoli cell differentiation [10] and in chondrogenesis [11], SOX9 is now recognised as a critical element for the development of many other organs such as the central nervous system [12], the lung [13], the pancreas [14], the hair follicles [15], the retina [16], the kidney [17], the heart [18], the liver [19] and the prostate [20] (for review see Ref. [21]). SOX9 has also been involved in the tumorigenesis of many of these organs, as for the prostate [22,23], the skin [24,25], the glia [26], the ovary [27], the lung [28], the breast [29,30], the liver [31,32] and the pancreas [33,34]. The present review focusses on the dose-dependent effect of SOX9 in the intestine epithelium and on its incidence on colorectal cancer (CRC).

2. The dose-dependent effect of SOX9 in the intestine epithelium

Historically, we first observed that SOX9 is selectively expressed at the bottom of the crypts where intestine epithelial cells are actively proliferating in response to the activated canonical Wnt/β-catenin signalling pathway [35]. We further established both *in vitro* and *in vivo* that SOX9 is a target gene of this oncogenic signalling pathway: we demonstrated that the β-catenin/transcription factor 4 (TCF4) transcription factor complex is required for SOX9 expression in intestine epithelial cells, and we observed that the SOX9 protein is not detected in the intestine epithelium of Tcf4-deficient neonate mice [35]. However, despite SOX9 being a target gene of the oncogenic Wnt/β-catenin pathway, we [36] and others [37] observed that a *Sox9* knock out targeted in the intestine epithelium results in an increased proliferation of both the small intestine and colon epithelial cells and associates with the occurrence of multiple micro-adenomas. We interpreted the resulting phenotype as the consequence of the loss of the SOX9 dependent retro-control on the activity of the Wnt/β-catenin signalling since SOX9 is not only a target but also a strong inhibitor of the Wnt/β-catenin signalling pathway [36] (Fig. 1). In addition, mice with a *Sox9* knock out targeted in the intestine epithelium lack Paneth cells, exhibit proliferating cells instead and display a decrease of goblet cells. Thus, SOX9 is up regulated by the Wnt/β-catenin pathway and likely promotes the secretory lineage. Conversely, SOX9 is down regulated by the Notch pathway that promotes the absorptive enterocyte lineage [38].

Using an elegant *Sox9* enhanced green fluorescent protein transgenic mice model, Formeister *et al.* further evidenced a bimodal role for SOX9 in intestine epithelial cells such that low SOX9 expression supports proliferation whereas high level of SOX9 suppresses proliferation [39]. More recently Roche *et al.* postulated that SOX9 dosage controls the dynamic inter-conversion between actively proliferating and reserve intestinal stem cell (ISCs) states [40] (Fig. 2A). Indeed, the authors demonstrated that, in homeostatic conditions, actively proliferating ISCs convert to reserve ISCs state via an up-regulation of SOX9 while secretory progenitor cells remain quiescent upon high doses of SOX9 and will differentiate into Paneth cells with time (Fig. 2B). By contrast, when actively proliferating ISCs are damaged upon radiation, reserve ISCs reduce SOX9 expression to doses suitable for cell proliferation and with acquisition of an actively proliferating ISC state (Fig. 2C). Together, these data provide evidence for a dose-dependent effect for SOX9 in the intestine epithelium and further provide an explanation of SOX9 requirement for differentiation into Paneth cells (Fig. 3, Roche *et al.* model).

3. Evidence for SOX9 tumour suppressor activity in the intestine epithelium

In addition to the anti-proliferative activity described for SOX9 *in vivo* [36,37], several recent lines of evidence further argue for a tumour suppressor activity for SOX9 in the intestine epithelium.

I. SOX9 overexpression in human or murine CRC cells is sufficient to inhibit cell proliferation [6,41] whereas SOX9 knock down increases the proliferation of the human HT29 CRC cells [41].

II. SOX9 knock out in the intestine epithelium correlates with an increase of tumour burden in an $Apc^{Min}/^{+}$ context [41] whereas a doxycycline-inducible