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HIGHLIGHT

SOX17-mediated immune evasion in early colorectal cancer: From pre-malignant adaptation to tumor progression

KEY WORDS

Immune escape;
Tumor microenvironment;
Immunotherapy;
Immunosuppressive tumor microenvironment;
Transcription factor;
$CD8^+$ T cell infiltration;
AKP organoids;
CRISPR−Cas9 genome editing

One of the hallmarks of cancer is its inherently immunosuppressive microenvironment, which strategically manipulates surrounding immune cells, signaling molecules, and structural components to shield cancer cells from immune attacks and foster tumor progression[1]. Such tumor microenvironment is characterized by the presence of immunosuppressive entities such as tumor-associated macrophages, T cells, tumor-associated neutrophils, and myeloid-derived suppressor cells (MDSCs), as well as metabolic alterations like hypoxia[2] and elevated lactate levels[3]. Advancements in understanding and targeting this microenvironment have opened up new possibilities for more effective cancer diagnoses[4,5] and treatments[6−9]. Immunotherapy, programmed to boost the immune system's capacity to identify and kill cancer cells, offers a highly specific and potentially enduring therapeutic alternative[10]. In contrast to conventional therapies such as chemotherapy and radiation, immunotherapy specifically targets and destroys cancer cells, minimizing damage to normal cells and reducing adverse side effects.

Although the immunosuppressive tumor microenvironment has been extensively studied in locally advanced and metastatic cancers, less is understood about the mechanisms through which pre-cancerous and early invasive tumors evade immune surveillance. Recently, a collaborative work led by the Massachusetts Institute of Technology (MIT) and the Dana−Farber Cancer Institute, published in *Nature* under the title "SOX17 enables immune evasion of early colorectal adenomas and cancers" pinpointed SOX17 as a transcription factor that is induced during the initial stages of colorectal cancer (CRC) tumorigenesis and plays a crucial role in enabling tumor initiation and progression by inhibiting immune clearance (Fig. 1)[1]. This study highlights the ways in which malignant tumors circumvent immune surveillance to orchestrate an immunosuppressive microenvironment at the pre-cancerous or early stages, offering significant insights for enhancing current immunotherapies.

Briefly, the authors engineered mouse CRC organoids containing *Apc*, $Kras^{G12D}$ and *Trp53* mutations (termed 'naïve AKP organoids') using CRISPR−Cas9 editing and Cre-mediated recombination, and transplanted them into immunocompetent mice to generate organoids from the primary tumors (called 'primary tumor-derived AKP organoids'), in order to understand the epigenetic evolution during CRC progression. Parallel *in vitro* and *in vivo* RNA sequencing and chromatin accessibility analysis revealed an enhanced fetal intestinal gene expression signature, indicating a reactivation of developmental genes in the primary tumor-derived organoids. SOX17 was identified as a key transcription factor regulating these transcriptomic and epigenetic modifications, exhibiting significantly upregulated expression in tumor-derived AKP organoids from both mouse and human colon cells. Additionally, SOX17 was demonstrated to enable tumors to evade immune detection during colonic tumorigenesis. To investigate further, the authors eliminated SOX17 expression in tumor-derived AKP organoids using CRISPR−Cas9 editing. The resulting SOX17-null organoids, when transplanted into immunocompetent mice, led to significantly fewer and smaller tumors, with enhanced immune infiltration of $CD4^+$ and $CD8^+$ T cells.

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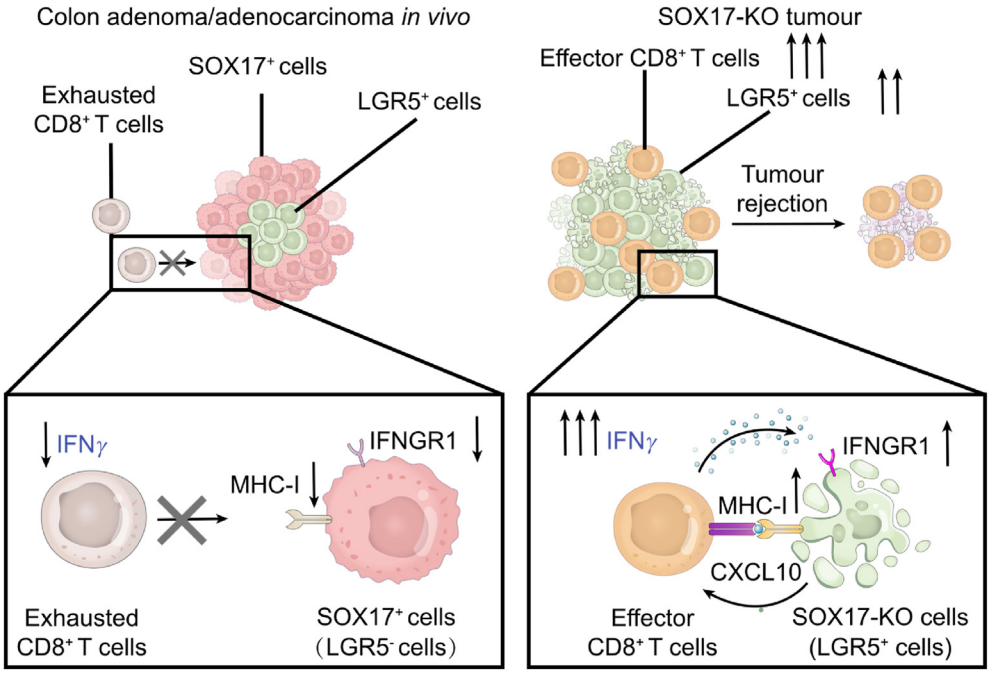

Figure 1 This highlight provides a concise overview of the recent study titled "SOX17 enables immune evasion of early colorectal adenomas and cancers" published in *Nature*, highlighting the pivotal role of SOX17 in immune evasion. Reproduced with permission from Ref. 1. Copyright © 2024, Springer Nature.

However, similar experiments conducted in immunodeficient mice showed consistent tumor development, suggesting that SOX17 facilitates tumor establishment and growth through suppression of anti-tumor immune responses.

To explore how SOX17 influences the tumor-associated immune environment in CRCs, the authors conducted single-cell RNA sequencing (scRNA-seq) on $CD45^+$ immune cells extracted from both control and SOX17-null tumors at various stages post-transplantation. Initial observations revealed a similar infiltration of myeloid cells in both tumor types, but as the study progressed, distinct differences in immune cell populations emerged. By the fourth week, the SOX17-null tumors displayed a robust presence of active $CD8^+$ T cells with potent anti-tumor effects, contrasting sharply with the control tumors, which showed signs of immune cell exhaustion. The critical role of $CD8^+$ and $CD4^+$ T cells in tumor rejection was further validated through depletion experiments, where the growth of SOX17-null tumors was notably reduced in the presence of these cells. Mechanistically, SOX17 inhibits the interferon-γ (IFNγ) signaling pathway, aiding tumor cells in evading immune detection and destruction. Specifically, SOX17 significantly downregulates the expression of *Ifngr1*, one of the subunits of the IFNγ receptor, by binding directly to the *Ifngr1* promoter. Consequently, SOX17 reduces the secretion of crucial T cell attractant chemokines such as *Cxcl10*, which hampers T cell recruitment, and suppresses *Ifngr1*-mediated major histocompatibility complex class I (MHC-I) expression, decreasing the recognition of tumor cells by $CD8^+$ T cells. In contrast, SOX17-null tumors exhibited enhanced IFNγ signaling, leading to increased MHC-I and CXCL10 expression, heightened immunogenicity, and improved T cell recruitment.

Indeed, SOX17 induction was observed even at the very earliest stages of tumorigenesis, notably during the formation of pre-malignant adenomas. Rather than using AKP organoids, the authors induced APC loss in intestinal stem cells to create *Apc*-null adenomas. These adenomas have the potential to progress to invasive colonic adenocarcinomas through the acquisition of additional mutations over time. Elevated levels of SOX17 were found in *Apc*-null adenomas compared to normal tissue, suggesting that the colonic environment triggers SOX17 expression during the pre-malignant phase. The pivotal role of SOX17 was further validated by simultaneously deleting both APC and SOX17, leading to a marked decrease in adenoma formation. This decrease was associated with an increased presence of $CD4^+$ and $CD8^+$ T cells, indicating that SOX17 facilitates adenomas in evading immune detection. Additional experiments demonstrated that depleting $CD8^+$ T cells in mice lacking both APC and SOX17 led to the resumption of tumor formation, emphasizing SOX17's critical role in protecting emerging tumors from immune-mediated destruction.

An additional study was conducted to evaluate SOX17 expression and its correlation with $CD8^+$ T cell infiltration in human adenomas and CRCs across various pathological stages (pT1 to pT4). The results indicated that all patient adenomas and early-stage CRCs (pT1) consistently showed high to moderate levels of SOX17 expression, accompanied by low $CD8^+$ T cell infiltration, mirroring findings in mouse models. In the more advanced stages of CRC (pT2 to pT4), although most tumors maintained high to moderate SOX17 expression, a significant subset exhibited reduced or absent SOX17 expression. Intriguingly, tumors with diminished or absent SOX17 expression demonstrated substantially higher $CD8^+$ T cell infiltration. This trend supports the hypothesis that robust SOX17 expression in early-stage adenomas and CRCs aids in the immune evasion of early dysplastic and cancerous human colorectal cells by limiting the presence of $CD8^+$ T cells.

Intriguingly, in *Apc*-null adenomas, SOX17 is predominantly expressed in the upper compartments of tumors, conspicuously absent from the crypt regions where $LGR5^+$ stem-like cells are located. The study found that SOX17 and LGR5 expressions are mutually

exclusive. Transplantation assays demonstrated the simultaneous presence of *Lgr5*$^{+}$SOX17^{-} and *Lgr5*$^{-}$SOX17^{+} cells within the tumors, illustrating the inherent cellular plasticity in CRCs. The autochthonous tumor models used in the study showed that deleting SOX17 or depleting CD8^{+} T cells increased the proportion of LGR5^{+} cells, underscoring SOX17's role in creating a less immunogenic tumor environment by promoting the prevalence of less immunogenic LGR5^{-} tumor cells. Additionally, the loss of SOX17 in adenomas led to an increased expression of MHC-I, pointing to its potential role as a transcriptional repressor of LGR5. This effect may be explained by the enforced expression of SOX17 distinguishing between fetal stem cells and their adult counterparts, and reactivating the fetal intestinal gene expression program in AKP organoids. This highlights its significant role in epigenetic reprogramming and promoting immune evasion by enhancing the survival of LGR5^{-} cells during the early stages of colon tumorigenesis.

This comprehensive study confirmed the role of SOX17 in triggering an immune evasion program that supports tumor initiation and progression during the pre-malignant stages of CRCs. It also demonstrated that the absence of SOX17 in tumor cells not only modifies the composition and activity of immune cells within the tumor microenvironment but also increases the tumor's vulnerability to immune clearance, by enhancing a more potent and effective anti-tumor immune response. These findings hold significant promise for early-stage colon cancer therapy by targeting and inhibiting SOX17. Understanding the pathway through which SOX17 operates enables the design of drugs that can specifically inhibit or modulate its activity, for example, by binding to SOX17 to block or disrupt its role in immune evasion. Consequently, this may restore the immune system's ability to recognize and destroy cancer cells.

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Author contributions

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Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Goto N, Westcott PMK, Goto S, Imada S, Taylor MS, Eng G, et al. SOX17 enables immune evasion of early colorectal adenomas and cancers. *Nature* 2024;**627**:636−45.
2. Yu WQ, Lin RY, He XQ, Yang XT, Zhang HL, Hu C, et al. Self-propelled nanomotor reconstructs tumor microenvironment through synergistic hypoxia alleviation and glycolysis inhibition for promoted anti-metastasis. *Acta Pharm Sin B* 2021;**11**:2924−36.
3. Shan YL, Ni Q, Zhang QX, Zhang MY, Wei B, Cheng LG, et al. Targeting tumor endothelial hyperglycolysis enhances immunotherapy through remodeling tumor microenvironment. *Acta Pharm Sin B* 2022;**12**:1825−39.
4. Xu Q, Pan YC, Liu XL, Gao YF, Luan XW, Zeng F, et al. Hypoxia-responsive platinum supernanoparticles for urinary microfluidic monitoring of tumors. *Angew Chem Int Ed* 2022;**61**:e202114239.
5. Gao YF, Wang YP, He BS, Pan YC, Zhou DT, Xiong MQ, et al. An enzyme-loaded metal−organic framework-assisted microfluidic platform enables single-cell metabolite analysis. *Angew Chem Int Ed* 2023;**62**:e202302000.
6. Feng SJ, Zhang Y, Gao YF, Liu YT, Wang YY, Han X, et al. A gene-editable palladium-based bioorthogonal nanoplatform facilitates macrophage phagocytosis for tumor therapy. *Angew Chem Int Ed* 2023;**62**:e202313968.
7. Li XQ, Pan YC, Chen C, Gao YF, Liu XL, Yang KY, et al. Hypoxia-responsive gene editing to reduce tumor thermal tolerance for mild-photothermal therapy. *Angew Chem Int Ed* 2021;**60**:21200−4.
8. Li ZK, Pan YC, Du SY, Li YY, Chen C, Song HX, et al. Tumor-microenvironment activated duplex genome-editing nanoprodrug for sensitized near-infrared titania phototherapy. *Acta Pharm Sin B* 2022;**12**:4224−34.
9. Pan YC, Luan XW, Zeng F, Wang XY, Qin SR, Lu QL, et al. Logic-gated tumor-microenvironment nanoamplifier enables targeted delivery of CRISPR/Cas9 for multimodal cancer therapy. *Acta Pharm Sin B* 2023;**14**:795−807.
10. Xu YY, Xiong JY, Sun XY, Gao HL. Targeted nanomedicines remodeling immunosuppressive tumor microenvironment for enhanced cancer immunotherapy. *Acta Pharm Sin B* 2022;**12**:4327−47.

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