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Dedifferentiation of Epithelial Cells Incorporates Immune Reprogramming

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Recent innovations in immunotherapies have cured cancers but remain ineffective in pancreatic cancer, which exhibits intertwined dynamics of dedifferentiation of epithelium and reprogramming of immunosuppression. Emerging evidence reveals the biological mechanisms through which dedifferentiation recapitulates immune reprogramming, providing new insights into the therapeutic potential of controlling dedifferentiation for sensitization to immunotherapies.

Dedifferentiation Dynamics

Differentiation is the cellular mechanism of one embryonic stem cell giving rise to fully functioning cells that develop into a multicellular organism. The differentiation process undergoes a strict programming sequence towards the biological commitment of cell identity. As a continuation, differentiation in adulthood presents when pluripotent stem cells divide and produce daughter cells, which then leave the cell cycle and become highly specialized cell types that fulfill a certain function, termed 'terminal differentiation'. Conversely, dedifferentiation is a distinct cell reprogramming state that reverses the cell trajectory in its lineage. Dedifferentiated cells usually gain self-renewal capacity and proliferative activity and, in the extreme, become immortalized or gain stemness. Endogenous dedifferentiation, such as wound healing, can operate to trigger redifferentiation of progenitor cells into specialized cells, and it is commonly recognized in regenerative medicine as having protective potential for repairing damage from environmental stress. However, stresses, such as massive inflammatory stimulation or oncogenic signaling cascades [1], can drive dedifferentiated cells to tumorigenesis, followed by varying degrees of oncogenic dedifferentiation [2] and cancer immunoediting [3].

After the escape phase of the cancer immunoediting process has occurred [3], design of antitumor therapies can be quite challenging and prompts us to refine our understanding of the interface between tumor cells and the immune microenvironment. Here, we hypothesize that dedifferentiation of epithelial cells recapitulates immune reprogramming and communication. Complementary evidence shows that dedifferentiated tumor cells {EHF [ETS (E26 transformation-specific) homologous factor] deficiency [4,5] or ectopic expression of forkhead box protein 3 (FOXP3) [6,7]} induce a substantial dysfunction of immune regulation (Figure 1A–C). Additional experimental findings underscore that tumor-derived immune molecules [transforming growth factor (TGF)β1 [5], granulocyte-macrophage colony-stimulating factor (GM-CSF) [5], interleukin (IL)-35 [8,9], C-C motif chemokine ligand 5 (CCL5) [7,8], and IL-37 [10]] modulate the signaling pathways of epithelial cells, especially cell dedifferentiation, along with communication with stromal cells (Figure 1D–G). In the current context of immunology, the conceptualization of the immunosuppressive role of dedifferentiation suggested by these cell biology findings opens new possibilities for targeted therapy and immunotherapy.

EHF Deficiency Shapes Dedifferentiation and Immunosuppression

Dedifferentiation of pancreatic acinar and ductal cells was suspected to be one possible genesis of ductal adenocarcinoma in the pancreas, which was then validated [1] by *in vivo* lineage-tracking methods. Dedifferentiation to mesenchymal reprogramming causes aggressive malignant behavior of pancreatic ductal adenocarcinoma (PDAC), characterized by increased proliferation, metastatic dissemination, and poor prognosis [1]. An essential transcription factor, EHF, is found to be fully engaged in terminal differentiation, using DNA methyltransferases DNMT3A and DNMT3B to prime gene induction [11]. Recent evidence shows that EHF deficiency in PDAC drives dedifferentiation [4] and orchestrates tumor-derived TGFβ1 and GM-CSF production, which subsequently induce the conversion and expansion of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment [5] (Figure 1D). In addition, EHF deficiency is found to mediate mesenchymal and stem-like properties in prostate epithelium. Notably, EHF serves as an independent predictor of survival for PDAC, as a tumor suppressor gene, as a gatekeeper of differentiation in epithelial–mesenchymal transition [4], and as a biomarker for anti-programmed cell death protein 1 (PD-1) efficacy [5]. At first glance, this crosstalk of tumor-derived TGFβ1 and GM-CSF production might appear to be merged into the concept of tumor intrinsic immunosuppression [3]. However, this immunosuppression is preceded by EHF deficiency associated with dedifferentiation and diminishes when EHF is overexpressed. Unfortunately, inducing EHF expression to drive dedifferentiated cells to terminal differentiation remains a challenge for therapeutic design. As an alternative, a series of personalized therapies targeting immunosuppressive mechanisms may be feasible yet laborious. Further understanding of the upstream mechanism in EHF production is urgently required for eliminating dedifferentiation and immunosuppression altogether.

Ectopic Expression of FOXP3 Mediates Dedifferentiation and Immune Reprogramming

Resembling the effect of EHF deficiency, ectopic expression of FOXP3 in epithelial

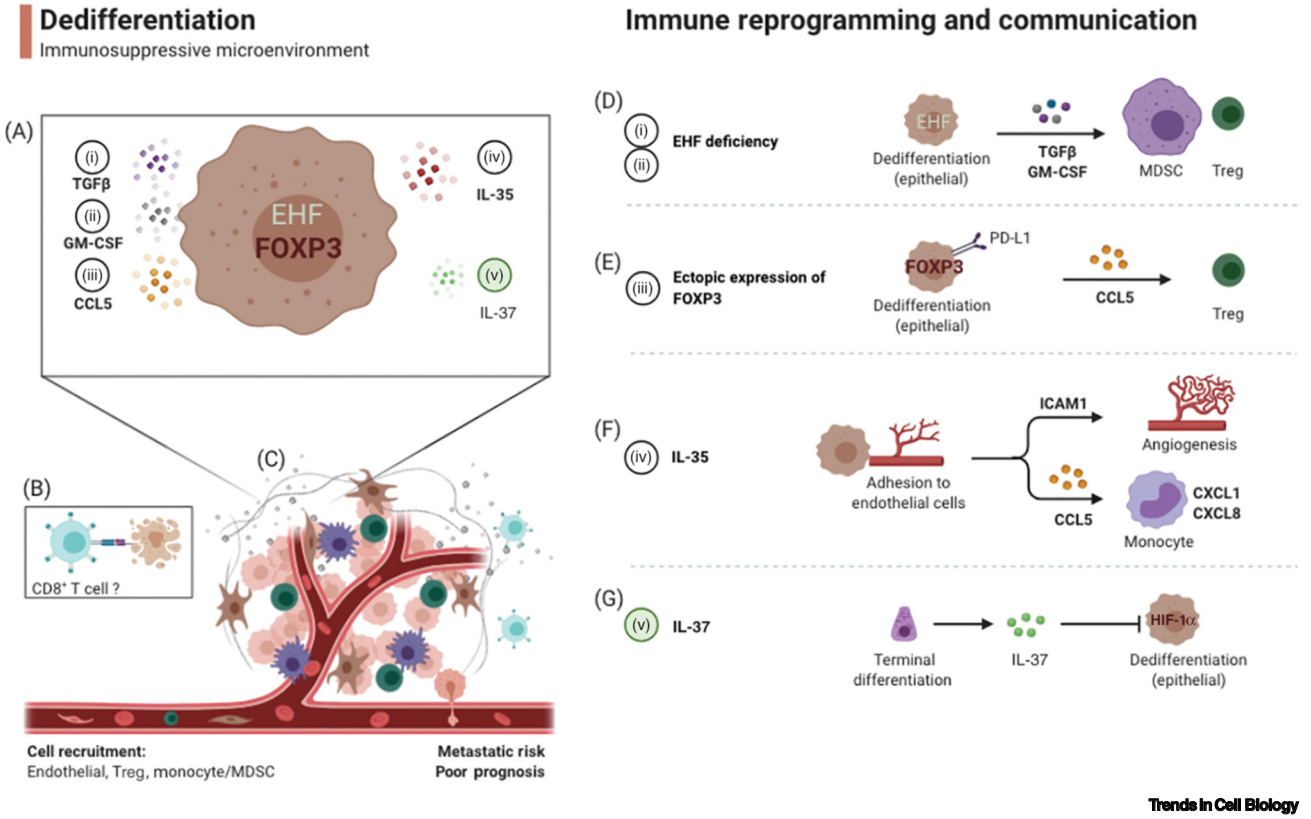

Figure 1. Dedifferentiation of Epithelial Cells Incorporates Immune Reprogramming and Induces an Immunosuppressive Microenvironment. (A) Dedifferentiation of epithelial cells led by EHF deficiency or ectopic FOXP3 expression recapitulates immune communication by inducing (i) TGFβ, (ii) GM-CSF, (iii) CCL5, and (iv) IL-35 production but (v) a reduction in IL-37. (B) The $CD8^+$ effector T cell-mediated antitumor response is impaired in the dedifferentiation-induced immunosuppressive microenvironment. (C) The oncogenic dedifferentiation-mediated immunosuppressive microenvironment, generated via recruiting endothelial cells, Tregs, monocytes, and MDSCs but not $CD8^+$ effector T cells, results in increased metastatic risk and poor prognosis. (D–G) Dedifferentiation triggers a variety of immune reprogramming and communication. (D) (i and ii) In EHF deficiency, TGFβ and GM-CSF recruit and expand MDSCs and Tregs. (E) (iii) With ectopic expression of FOXP3, upregulation of PD-L1 mediates $CD8^+$ T cell exhaustion, and secreted CCL5 recruits Tregs. (F) (iv) IL-35 increases adhesion to endothelial cells to promote angiogenesis, and secreted CCL5 polarizes monocytes to produce CXCL1 and CXCL8. (G) (v) IL-37 from differentiated cells suppresses HIF-1α expression in dedifferentiated cells. Abbreviations: CCL5, chemokine ligand 5; CXCL1, C-X-C motif chemokine ligand 1; CXCL8, C-X-C motif chemokine ligand 8; EHF, ETS (E26 transformation-specific) homologous factor; FOXP3, forkhead box protein 3; GM-CSF, granulocyte–macrophage colony-stimulating factor; HIF-1α, hypoxia-inducible factor-1 α; ICAM1, intercellular adhesion molecule 1; IL, interleukin: MDSCs, myeloid-derived suppressor cells; PD-L1, programmed death-ligand 1; TGFβ, transforming growth factor β; Tregs, regulatory T cells.

lineage is associated with dedifferentiation [6] and recruits Tregs and MDSCs [7]. The transcription factor FOXP3 is a master regulator of Treg function and defines the lineage specificity of Tregs and their epigenetic identity [12]. FOXP3 expression in epithelial lineage has been observed in several tumor types, but cancer-FOXP3 (c-FOXP3) plays cell-type-dependent roles in molecular pathogenesis. Serving as an oncogene in PDAC, c-FOXP3 overexpression promotes tumor progression in immunocompetent mice but not in immunocompromised or in Treg-depleted conditions [7]. Further evidence [7] suggests that c-FOXP3 transactivation of CCL5 secretion from dedifferentiated PDAC cells induces immunosuppressive reprogramming via Treg recruitment. Furthermore, c-FOXP3 directly activates programmed death-ligand 1 (PD-L1) expression-mediated $CD8^+PD\text{-}1^+$ T cell exhaustion by an innate mechanism involving c-FOXP3 binding to motif-a of the PD-L1 promoter, other than adaptive immune resistance via interferon signaling (Figure 1E) [7]. Such dual suppressive capacities of c-FOXP3 suggest that dedifferentiation of PDAC cells recapitulates Treg crosstalk in remodeling immune escape. On targeting nonredundant suppressive immune reprogramming and communication, the combination of anti-CCL5 and anti-PD-L1 antibodies enables effective antitumor immune responses to eliminate $c\text{-}FOXP3^{high}$ dedifferentiated malignant cells [7].

Tumor-Derived Immune Molecules in Remodeling the Microenvironment

A few tumor-derived immune molecules have been identified as operating signaling pathways of epithelial cell dedifferentiation and communication in addition to

their typical roles in immunosuppression (Figure 1D–G). As discussed earlier, dedifferentiated cell-derived TGFβ1 [5], GM-CSF [5], and CCL5 [7,8] are essential for Treg and MDSC accumulation and are marked as the old triad of immunosuppressive cytokines in PDAC progression. Recently, two distinct cytokines (IL-35 [8,9] and IL-37 [10]) have been characterized as being involved in the trajectory of dedifferentiation and immunosuppression.

IL-35 is an anti-inflammatory cytokine of the IL-12 family produced mainly by Tregs or regulatory B cells (Bregs). IL-35 binds to its receptors GP130 and IL-12Rβ2 and induces phosphorylation of signal transducer and activator of transcription (STAT) 1 and STAT4 and nuclear translocation of phosphorylated (p)-STAT1 and p-STAT4, respectively. Tumor-derived IL-35 and IL-35R are required for colocalized intercellular adhesion molecule 1 (ICAM1) overexpression in PDAC cells and mediate epithelial adhesion to endothelial cells through an ICAM1–fibrinogen–ICAM1 bridge [9]. This stimulation of ICAM1 occurs solely through the GP130–GP130 homodimer on the STAT1 branch of the angiogenesis cascade (Figure 1F). GP130, but not IL-12Rβ2, is consistently associated with increased metastatic risk and poor clinical outcome [9]. Interestingly, in the immunosuppressive feedforward loop of recruiting and polarizing monocytes to produce CXCL1 and CXCL8, IL-35 induces CCL5 transcription by binding to the GP130–IL12Rβ2 receptor through heterodimerization of pSTAT1–pSTAT4 [8]. Furthermore, neutralizing IL-35 prevents monocyte infiltration and sensitizes gemcitabine chemotherapy in a PDAC animal model (Figure 1F), suggesting the need for therapeutic options that disrupt extracellular communication and target the autocrine or paracrine secretion of IL-35 from dedifferentiated tumor cells.

IL-37, however, notably decreases in dedifferentiated PDAC cells. Originating from hematopoietic cells, IL-37 appears to be a natural inhibitor of innate inflammatory responses [10]. Under uncontrolled stress, hypoxia-inducible factor (HIF-1α [10]) binds to hypoxia response elements (HREs) in the IL-37 promoter and attenuates IL-37 expression, which triggers subsequent events including dedifferentiation, tumor metastasis, vessel invasion, and chemoresistance in PDAC. Paradoxically, IL-37 dampens HIF-1α expression through STAT3 inhibition [10] (Figure 1G). Although knowledge of IL-37 is still limited, IL-37 could serve as an exciting target for reconstituting immune surveillance against oncogenic dedifferentiation.

Concluding Remarks

Taken together, these findings [4,5,7–10] highlight a linear biological circuitry where the ectopic expression of immune molecules in dedifferentiated epithelial cells incorporates the immunosuppressive microenvironment and tumor progression (Figure 1A–C). Dedifferentiated cells exerting cytokine communication in PDAC recapitulate the reprogramming mechanisms of Treg and innate immune cells, such as monocytes or MDSCs (Figure 1D–G). Thus, the ineffectiveness of immunotherapies is largely attributable to dedifferentiation-initiated immune reprogramming [4,5,7–10].

Admittedly, with limited understanding of the evolutionary variation of epithelial cells, some cell-type-dependent features uncovered in these studies may not fit every disease process ubiquitously; but, a bioinformatic discovery on the pan-cancer stemness landscape suggested the same association of dedifferentiation with immune content [2]. Along with deep learning on immune profiles, comprehensive validation on dedifferentiated epithelium in lesions from multiple organs will be required to establish the biological roadmap of dedifferentiation-driven immunosuppression.

Targeting dedifferentiated cells in cancer therapy is therefore of paramount importance in controlling tumor development and the immunosuppressive cascade. Future exploration of cytokine crosstalk, extracellular vesicle delivery, and epigenetic modification involved in the dedifferentiation process will enable us to decipher the determining triggers of the upstream mechanisms of dedifferentiation signaling, which could serve as the priming targets that drive cellular differentiation. Instead of killing dedifferentiated cells using cytotoxic chemotherapy, new therapeutic strategies aimed at training dedifferentiated epithelial cells to undergo terminal differentiation may harness the differentiation machinery to overcome the immunosuppressive tumor microenvironment and become sensitive to immunotherapies.

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Declaration of Interests

The authors declare no competing interests.

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