Doublecortin-Like Kinase 1

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Contributor: Parthasarathy Chandrakesan

Microtubule-associated doublecortin-like kinase 1 (DCLK1) is an accepted marker of tuft cells (TCs) and several kinds of cancer stem cells (CSCs), and emerging evidence suggests that DCLK1-positive TCs participate in the initiation and formation of inflammation-associated cancer. DCLK1-expressing CSCs regulate multiple biological processes in cancer, enhance resistance to host anti-tumor immunity, promote resistance to therapy, and are associated with metastasis.

Keywords: DCLK1,tuft cells,cancer stem cells,microenvironment,immunotherapies

1. Introduction

Microtubule-associated doublecortin-like kinase 1 (DCLK1) was originally thought to be a brain-specific protein before 2006[1] when Giannakis et al. first reported DCLK1 as a potential marker of stem-like cells of the small intestine[2]. However, further research has identified that these cells as differentiated tuft cells (TCs) possessing a variety of unique molecular and functional characteristics[3]. DCLK1+ tuft cells of the gastrointestinal tract are characterized by microvilli and may be long-lived and display self-renewal or progenitor functionality under some conditions[4][5][6]. Importantly, they regulate the immune microenvironment through IL-25/IL-17RB signaling in order to affect epithelial repair after injury, and may initiate inflammation-associated tumorigenesis after mutation [7][8][9][10][11][12]. In 2008, the Houchen group proposed that DCLK1 is a specific marker protein for intestinal adenoma stem cells [13], which brought attention to DCLK1 in cancer research and was the first of a series of research reports providing evidence that it might be an effective target for oncology drug development. To date, DCLK1 has been demonstrated to be a relatively selective marker of several kinds of cancer stem-like cells or cancer stem cells (CSCs) including in colon, lung, pancreas, kidney, and esophageal cancers[14][15][16][17]. After twenty years of research, DCLK1 is accepted as a specific marker of TCs and several kinds of CSCs, and is well known for its ability to regulate tumor growth, invasion, metastasis, epithelial-mesenchymal transition (EMT), pluripotency, angiogenesis, immuno-regulation and pro-survival signaling[18][19][20][21][22].

CSCs are an important subpopulation of cells in the immunosuppressive tumor microenvironment (TME), which in turn provides a niche to support stem cell characteristics including self-renewal, differentiation, and immunosuppressive cell recruitment. Tumors create an immunosuppressive microenvironment by secreting a variety of chemokines and cytokines which may recruit tumor associated macrophages (TAM), tumor associated neutrophils (TAN), myeloid derived suppressor cells (MDSC), and other regulatory immune cells. TAM and TAN differentiate from polarized macrophages and neutrophils respectively, and remodel the TME to support tumor growth and angiogenesis[23]. TAM have been shown to promote the degradation of extracellular matrix and secrete exosomes containing mRNA and miRNA which ultimately promote tumor invasion and metastasis. Both TAM and CD4+ T-cells secrete tumor necrosis factor alpha (TNF-α) and up-regulate NF-κB signal pathway to induce the expression of EMT transcription factors Snail and Twist[24]. Moreover, they enhance transforming growth factor-β (TGF-β) signaling to promote the self-renewal of CSCs[25]. Presently, CSCs are considered a key driver of chemotherapy resistance, recurrence, and metastasis. Recent work shows that DCLK1 promotes CSC self-renewal and drug-resistance and can be targeted to inhibit tumorigenesis in kidney cancer[26]. Furthermore, several recent studies show that DCLK1 affects tumor growth and metastasis via regulating TAM and immune checkpoint. Finally, monoclonal antibodies and chimeric antigen receptor T-Cells (CAR-T) based on DCLK1 have demonstrated potential as novel cancer immunotherapies[27][28][22].

2. Function of DCLK1-Expressing Gastrointestinal Tuft Cells

TCs are present above the +4 position of the intestinal crypt and in the villus where they function as a chemosensory and secretory cell type. Additionally, TCs are found in the respiratory tract, salivary gland, gallbladder, pancreatic duct, auditory tube, urethra, and thymus[29][30][31][32][33]. The majority within the intestinal epithelium express DCLK1, and accumulating evidence suggests that DCLK1+ tuft cells take part in a diffuse chemosensory system where they serve a sentinel function to detect chemical signals in the microenvironment and orchestrate the repair of local epithelial tissue [12]. For instance,

TCs located in the lung, colon and stomach epithelium can sense alterations to pH, nutrients, or the microbiota using taste receptors including GTP-binding protein α-gustducin and transient receptor potential cation channel subfamily M member 5 (TRPM5), or regulate capillary resistance to hypoxia by inducing an epithelial response via secretion of IL-25, leading to the activation of innate lymphoid type 2 cells (ILC2) and IL-13 secretion[34][35][36][37]. Using a transgenic intestinal epithelium specific DCLK1 knockout mouse model (Villin^{Cre};Dclk1$^{fl/fl}$), May et al. reported that DCLK1 deletion in tuft cells resulted in altered gene expression in pathways for epithelial growth, stemness, barrier function, and taste reception signaling further suggesting its importance in TCs[9]. Furthermore, several studies have reported that DCLK1-expressing TCs secrete various kinds of regulatory molecules such as leukotrienes, prostaglandins, nitric oxide and IL-25, which lead to ILC2 and LGR5+ stem cell-mediated tuft and goblet cell differentiation in chronic inflammation and injury[35][38]. This is a key emerging area of interest that will provide new knowledge about the inflammatory, pre-cancer, and tumor microenvironments as well as immune–tumor interactions as they relate to tumorigenesis and progression.

There is strong evidence that DCLK1 expression in TCs play an important functional role in epithelial repair processes of the gut. Intestinal epithelium-specific knockout of DCLK1 (Vil^{Cre};Dclk1$^{flox/flox}$) leads to increased severity of injury and death in mouse whole body irradiation and dextran sulfate sodium (DSS)-induced colitis models [6][9][39]. A recent study expounded on this idea more directly. Yi et al. reported the deletion of DCLK1 in the mucin-type O-glycan deficient model of ulcerative colitis (UC) resulted in greater severity of disease characterized by enhanced mucosal thickening and increased inflammatory cell infiltration. They found that in the absence of DCLK1, epithelial proliferative responses to chronic inflammation were impaired. However, the deletion of DCLK1 did not affect the numbers of intact TCs. These results indicate that DCLK1 expression is a regulator of TC activation status, despite not being involved in TC expansion[10]. Moreover, this function has consequences to the entire intestinal epithelial response to injury as supported by previous findings[6][9][39]. Although these findings are highly suggestive, further studies will be necessary to fully determine the exact mechanisms by which DCLK1 in TCs regulate this response.

DCLK1-expressing TC expansion has been observed in human Barrett's esophagus, chronic gastritis in transgenic mice, rat gastric mucosa and intestinal neoplasia[14][40][41]. While TCs are not usually proliferative, it appears that mutations acquired by stem cells or progenitors can be passed on to TCs, which might then interconvert into tumor initiating cells under inflammatory or injurious conditions. Alternatively, putative "long-lived" TCs might acquire and maintain mutations, finally initiating tumorigenesis after a secondary insult such as colitis[6]. During the early stages of tumorigenesis, DCLK1+ TC expansion is observed in the gastrointestinal niche where they interact with neurons and promote tumorigenesis by secreting acetylcholine to stimulate enteric nerves. Notably, intestinal epithelial cells can express acetylcholine receptors to activate Wnt signaling and regulate the differentiation of intestinal epithelial cells which may be required for tumorigenesis[42]. Using lineage tracing mouse models, Nakanishi et al. and Westphalen et al. concurrently demonstrated the DCLK1+ TC's cell-of-origin status in Wnt-driven tumorigenesis. In the Nakanishi study, the Apc$^{Min/+}$ model of intestinal polyposis was crossed with a Dclk1$^{Cre\text{-}ERT}$ mouse to generate lineage tracing (Apc$^{Min/+}$;Dclk1$^{Cre\text{-}ERT}$;R26^{LacZ}) and diptheria-toxin receptor TC-specific deletion (Apc$^{Min/+}$;Dclk1$^{Cre\text{-}ERT}$;iDTR) mice. Dclk1+ TC-based lineage tracing specifically traced the entirety of the adenoma in these mice. In comparison, an intestinal stem cell marker Lgr5-based model traced the entirety of the normal epithelium and the polyp. Moreover, deletion of DCLK1+ TCs using the diptheria-toxin receptor model resulted in a complete collapse of polyps within days[43]. The Westphalen study made use of an alternative Dclk1^{Cre} model which was crossed to an Apc$^{flox/flox}$ mouse. In this model, spontaneous tumorigenesis did not occur. However, lineage tracing experiments demonstrated a small, but abnormally long-lived, population of DCLK1+ TCs in the intestinal epithelium. In conditions of colitis induced chemically via DSS, these long-lived TCs gave rise to tumors with a severe adenocarcinoma-like phenotype[6]. Importantly, this study was the first to ascertain the existence of multiple functionally unique populations of TCs. This finding has now been confirmed by single-cell RNA-Sequencing studies which identified a separate immunomodulatory population of TCs[44].

In summary, DCLK1-expressing TCs play an important role in stimulating gastrointestinal epithelial stem cells in the microenvironment and contributing to cancer progression [45]. Moreover, studying the two distinct subpopulations of TCs separately may clarify their dual-role in epithelial restitution and tumorigenesis. Promisingly, specific markers for each TC subtype have already been identified[44]. Finally, limited evidence suggests that DCLK1-expressing intestinal TCs in the gut can promote tumor progression in hepatocellular carcinoma (HCC) through activating alternative macrophages in tumor microenvironment via secreting IL-25[46]. This distant signaling functionality across the gut-liver axis adds an interesting new dimension to understanding the role of TCs.

3. Function of DCLK1+ Acinar and Tuft Cells in Pancreatitis and Pancreatic Cancer

In the pancreas, DCLK1 is a marker of a population of pancreatic cancer-initiating cells, some of which have morphological and molecular features of gastrointestinal TCs[47]. However, DCLK1 also notably marks pancreatic acinar cells, which are a likely source of tumorigenesis through the acinar-ductal metaplasia process. Genetic lineage tracing experiments show that Dclk1+ pancreatic epithelial cells are necessary for pancreatic regeneration following injury and chronic inflammation. Moreover, KRAS mutation in Dclk1+ pancreatic epithelial cells leads to pancreatic cancer in the presence of induced pancreatitis[43]. In pancreatic tumors, it has recently been shown that immune cell-derived IL-17 regulates the development of TCs via increased expression of DCLK1, POU domain class 2 transcription factor 3 (POU2F3), aldehyde dehydrogenase 1 family member A1 (ALDH1A1), and IL17RC[48]. Intriguingly, DCLK1 kinase inhibitor can inhibit DCLK1+ organoids derived from pancreatic ductal adenocarcinoma patient tumors, indicating that DCLK1 activity and perhaps DCLK1+ TCs or acinar cells are a potential target for pancreatic ductal adenocarcinoma[49]. Interestingly, Chandrakesan and his team (2020)[22] have demonstrated that DCLK1+ pancreatic tumors educated M2-macrophage inhibit CD8+ T-cells proliferation and Granzyme-B activation. Inhibition of DCLK1 in these tumors in an organoid co-culture system enhanced CD8+ T-cell activation and associated organoid death. DCLK1+ tumors are the novel initiator of alternate macrophage activation that contributes to the immunosuppression observed in the PDAC TME. DCLK1+ pancreatic tumors may be an attractive target for PDAC therapy either alone or in conjunction with immunotherapeutic strategies. Although the underlying signaling mechanisms of DCLK1+ epithelial cell-mediated tumorigenesis require further elaboration, DCLK1 and DCLK1+ epithelial cells such as TCs are likely to be a target for new classes of immunotherapies and TME-remodeling drugs in gastrointestinal tract cancers.

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