Illuminate TWEAK/Fn14 pathway in intrahepatic cholangiocarcinoma: Another brick in the wall of tumor niche

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Conflict of interests: None to declare

Word count: 1499

Figure: 1

Conflict of interest statement: None

Financial support: None

Author contribution: LFa, MC and LFo contributed equally to the review

More than 20 years ago, a new secreted ligand of the tumor necrosis factor (TNF) family was identified in mouse peritoneal macrophage, human tonsil and fetal liver. However, in contrast to TNF, TWEAK induced only weakly apoptosis in a panel of cell types such as hematopoietic cells, fibroblasts and cancer cells, and was thus named TWEAK for “tumor necrosis factor-like weak inducer of apoptosis”. TWEAK displays two forms, membrane-bound or soluble. As TNF, TWEAK activates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway and thereby inflammatory responses. Meanwhile, and independently of TWEAK, fibroblast growth factor-inducible 14 (Fn14), a type I transmembrane protein was identified as a modulator of fibroblast adhesion and migration. Soon after, it was discovered in endothelial cells (ECs) for its role in angiogenesis as the receptor of TWEAK. While Fn14 was already known for its hepatic functions including regeneration and cancer, i.e. hepatocellular carcinoma (HCC) [1], the role of TWEAK/Fn14 signaling in cholangiocarcinoma (CCA), the second more frequent liver cancer after HCC, was unknown so far. In an elegant work, Dwyer et al. [2] highlighted essential functions of TWEAK/Fn14 in intrahepatic CCA (iCCA), the anatomical subtype of CCA arising above the second-order bile ducts. In iCCA, TWEAK promotes the development of the tumor niche sustaining tumor initiation and progression, which could pave the way for a potential treatment of iCCA patients since TWEAK/Fn14 pathway is a “druggable” target [1].

In normal adult liver, TWEAK and its receptor Fn14 are expressed at very low levels [3]. Fn14 expression is limited to blood vessels, in smooth muscle cells of the arterial wall, and bile ducts [3]. In iCCA, Dwyer et al. [2] found an increased expression of TWEAK and Fn14. Fn14 is upregulated more specifically in malignant cholangiocytes and correlates with well-differentiated tumors. Besides tumor cells, cells of the tumor microenvironment (TME) express Fn14, in particular cancer-associated fibroblasts (CAF). Expression of Fn14 in myofibroblasts had been already reported in fatty liver disease, i.e., non-alcoholic steatohepatitis, and in immune-mediated liver diseases, i.e., primary sclerosing cholangitis [3], indicating that one major target of TWEAK in liver diseases including CCA is myofibroblast. In contrast with Fn14,

TWEAK is barely expressed by tumor cells, whereas it is abundant in immune cells, mainly tumor-associated macrophages (TAM), identified as the main cell source of TWEAK, with little contribution of EC and CAF. Thus, in iCCA, TWEAK mostly originates from TME with preferential targets comprising tumor cells and CAF. Thanks to several iCCA mouse models, the authors investigated cholangiocyte responsiveness to TWEAK by evaluating the expression kinetic of Fn14 through CCA development. Of note, Fn14 was expressed also by cholangiocytes lining dysplastic biliary lesions, and as malignancy developed, Fn14 expression increased in tumor but not in non-malignant cholangiocytes. These observations hint at the possibility that TWEAK/Fn14 pathway is functionally active since the earliest steps of tumorigenesis, before the activation of other morphogens relevant in the malignant shift, such as Wnt and Notch [4]. A fundamental question that will need to be explored further is how *Fn14* is regulated during the course of CCA development. It is known that *Fn14* promoter contains a CpG island close to the transcription start site, and multiple consensus DNA sequences for transcription factors involved in oncogenic transformation including activator protein 1 (AP-1) and specificity protein 1 (Sp1) [5].

The TWEAK/Fn14 signaling is crucial for several aspects of iCCA biology. TWEAK is regarded as a tumorigenic factor since it stimulates proliferation and migration in malignant cells [6]. Two out of four human iCCA cell lines tested in Dwyer's study proliferate upon TWEAK stimulation although they all express Fn14, and display a robust activation of canonical and non-canonical NF-ΚB pathway in response to TWEAK. The mitogenic action is a well-established effect of TWEAK, which has been already described both in chronic liver diseases, where TWEAK regulates proliferation of hepatic progenitor cells [7], and in HCC [1]. With respect to cell migration, this has not been tested in Dwyer's study.

However, functionality of TWEAK goes far beyond the mitogenic effects, as it stimulates the secretion of a range of pro-inflammatory mediators in human iCCA cells. Among them, monocyte chemoattractant protein-1 (MCP-1, also known as CCL-2), interleukin (IL)-8, and

granulocyte macrophage-colony stimulating factor (GM-CSF) are secreted in cell culture medium. Nevertheless, mass spectrometry analysis of conditioned medium of iCCA cells treated with TWEAK revealed the presence of secreted factors regulating innate immune response, angiogenesis and extracellular matrix (ECM) remodeling, suggesting a wide role of TWEAK in building-up the tumor niche.

A fundamental facet of the NF-ΚB-regulated pro-inflammatory profile induced by TWEAK is the twofold effect exerted on macrophages. First, CCA cells up-regulate MCP-1 signaling to C-C chemokine receptor type 2 $(CCR2)^{+}$ cells to recruit circulating monocytes in the tumor niche. In CCA, previous studies showed that bone marrow-derived monocytes can be recruited by several mediators released by both tumoral and stromal cells, including CSF-1, vascular endothelial growth factor (VEGF)-A, and cytokines (IL-1β, IL-4, IL-8, IL-10, and IL-13), acting through common regulatory pathways (Notch, IL-6/STAT3, PI3K) [8]. In Dwyer's study, MCP-1 was the most prominent soluble factor secreted *in-vitro* by cultured iCCA cell lines exposed to TWEAK, much greater than GM-CSF and IL-8. This finding is of paramount importance since it addressed for the first time the role of MCP-1 in macrophage accumulation in iCCA. After directing trafficking of $CCR2^{+}$ monocytes to tumor niche, TWEAK-inducible factors released by CCA cells polarize them into a TAM, M2-skewed phenotype expressing CD206 along with a variety of cyto/chemokines, growth factors and receptors (MCP-1, IL-6, TNF, VEGF-A, and macrophage receptor with collagenous structure (MARCO)). Of note, MCP-1 secretion by the macrophages lock them in a mechanically self-sustaining feedforward loop further supporting macrophage gathering within the niche. A recent study showed that chemoattraction of circulating monocytes and their differentiation into TAMs was orchestrated by a specific subgroup of tumor cholangiocytes, as part of a repertoire of stem-cell like properties, and was mediated by IL-13, IL-34, and osteoactivin, in line with the existence of a highly specialized tumor niche in CCA. At this level, TAMs expressed both M1 and M2 phenotypes, displaying strong tumor-permissive activities *in-vivo* [9]. In iCCA, TAM accumulation associated with increased metastasisation and poor prognosis after surgery [10],

consistent with their ability to incite a more aggressive tumor phenotype. Pro-invasive functions of TAM involve multiple traits, including tumor cell proliferation, chemoresistance, angiogenesis, and immune escape (Figure 1).

As aforementioned, Dwyer and coll. found expression of Fn14 also in a subset of CAF, which respond to TWEAK by increasing cell proliferation and collagen deposition upon NF-KB activation. Accordingly, TWEAK effects had been investigated by single cell analysis in cirrhotic patients, where macrophage-derived TWEAK stimulated proliferation of $Fn14^+$ myofibroblasts [11]. Although the tumor-promoting effects exerted by CAF are multifaceted as shown even at the transcriptomic level [12], it would be tempting to speculate that the subset of $Fn14^+$ CAF dwelling in the iCCA niche might play more specific functions proficient to tumor initiation and progression, for instance by enhancing expression of cancer stem cell traits [13].

The TWEAK/Fn14 signaling can influence other functions of the iCCA niche relevant for tumor progression. By analyzing enriched gene ontology terms, Dwyer and coll. found TWEAK-inducible molecules associated with “ECM organization” and “blood vessel development”. Besides the abovementioned up-regulated expression of VEGF-A in macrophages patterned by TWEAK-inducible factors from iCCA cells, Dwyer reported Fn14 expression by EC in rodent and human iCCA, consistent with the capability of TWEAK to directly stimulate proliferation of EC, which Fn14 expression is up-regulated by VEGF-A and FGF-2 [14]. Noteworthy, the pro-angiogenetic functions of TAM molded by TWEAK-stimulated iCCA cells are sustained by VEGF-A but not VEGF-C expression, the main driver of tumor-associated lymphangiogenesis, which remains a prerogative of CAF [15]. Dissecting further the effects exerted by TWEAK/Fn14 on ECM and angiogenesis in tumor niche is a research area deserving attention by future studies.

The actions of TWEAK/Fn14 pathway on macrophage recruitment and polarization, and additionally, on CAF proliferation, provide interesting targets for possible therapeutic

intervention in iCCA, further corroborated by the relevant proportion of $Fn14^+$ cells in both tumor and CAF compartment (42.5 and 62.64%, respectively). Taking a dual approach of TWEAK/Fn14 targeting, based on pharmacological antagonism of the downstream effector MCP-1 in xenograft tumors and genetic ablation of Fn14 in transgenic mice upon carcinogenic treatment with thioacetamide, the authors obtained a significant inhibition of tumorigenesis associated with reduced macrophage infiltration. Although of great translational relevance, to date, strategies targeting the TWEAK/Fn14/MCP-1 axis are very preliminary, and only five clinical trials aimed at MCP-1 blockade have been recorded so far in liver tumors (all in HCC), with three of these still enrolling patients. TWEAK targeting by a monoclonal antibody (RG7212) has been evaluated in $Fn14^+$ advanced cancers in a phase I-first-in-human study, with effects on tumor proliferation and MCP-1 serum concentrations dependent on the expression levels of Fn14 [16]. Nevertheless, therapeutic opportunities derived from Dwyer's study are fascinating, because they provide tools to target the niche of iCCA, the "war room" whereby the tumor fate is decided.

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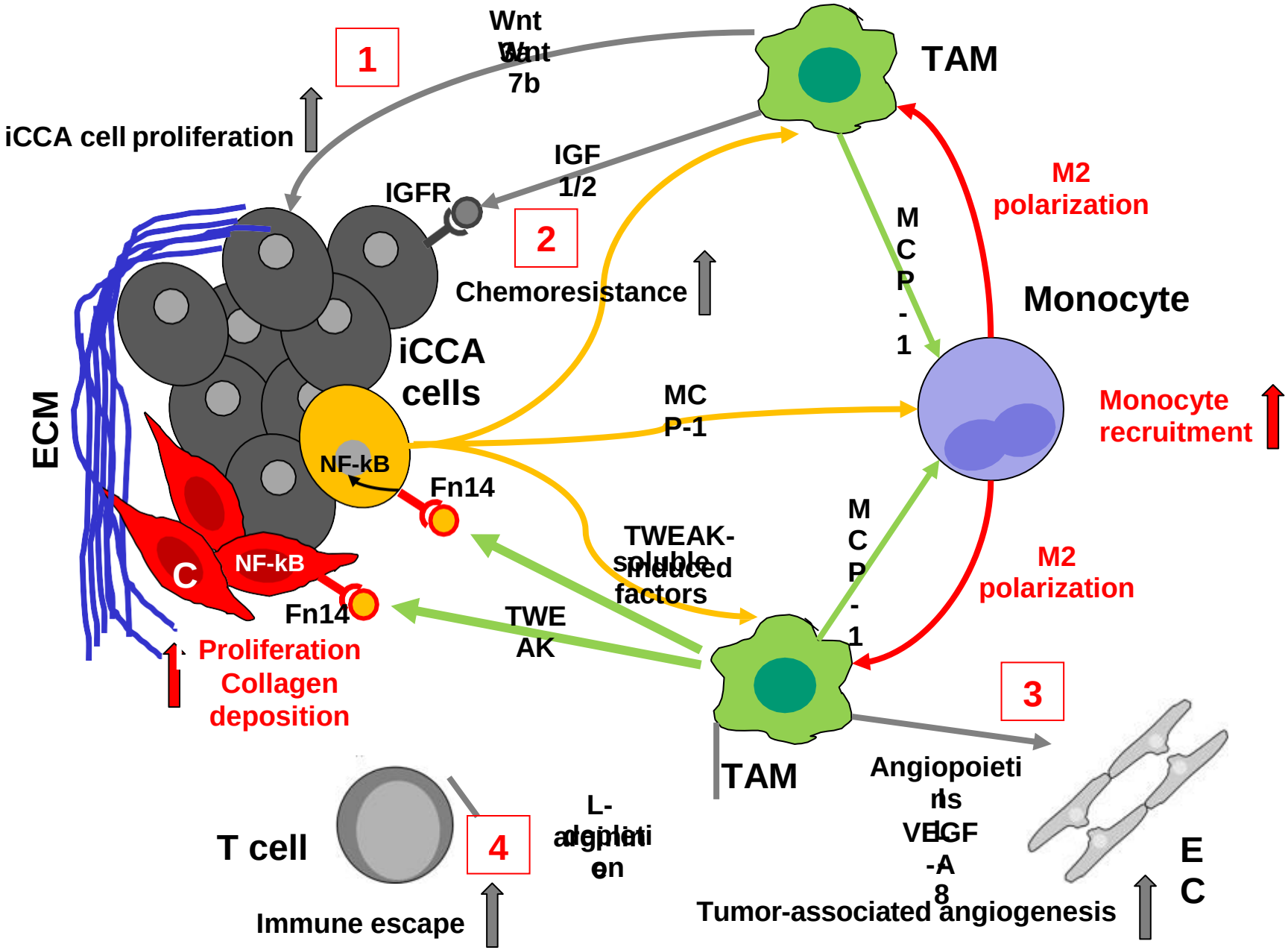
Figure legend

Role of the TWEAK/Fn14 pathway in the generation of the tumor niche in intrahepatic cholangiocarcinoma (iCCA). TWEAK released in the tumor microenvironment (bold green arrows), mostly by macrophages populating the tumor microenvironment, signals to Fn14 expressed by malignant cholangiocytes in the tumor niche (yellow cells) and by cancer-associated-fibroblasts (CAF, red cells), acting through the NF-ΚB pathway. Upon TWEAK stimulation, CCA cells produce MCP-1/CCL2, which promote recruitment of circulating monocytes (purple cells) and other factors (yellow arrows), which polarize them into a tumor-associated macrophage (TAM), M2-skewed phenotype (green cells). On the other hand, TWEAK-stimulated CAF start to proliferate and to secrete extracellular matrix (ECM) components (blue lines). Once patterned to the M2 phenotype, the TAM themselves produce MCP-1, which enforces monocyte chemoattraction (green arrows). Moreover, TAM may influence several pro-invasive functions of the tumor niche (grey colors). By releasing Wnt3a and Wnt7b, TAM activate canonical Wnt/β-catenin signaling leading to tumor cholangioycte proliferation [17]. By producing insulin-like growth factors (IGF) 1 and 2, which activate insulin/IGF receptors (IGFRs) on ductal epithelial cancer cells, TAMs may dampen the efficacy of cytotoxic drugs, as shown with gemcitabine in rodent models of pancreatic ductal adenocarcinoma [18]. Importantly, expression of insulin/IGFRs and their pathogenetic relevance for chemoresistance has been pinpointed also in iCCA cells [19]. By secreting VEGF-A, angiopoietin, and IL-8, TAMs cooperate to tumoral angiogenesis, and indeed Dwyer et al. reported increased VEGF-A expression in macrophages patterned by TWEAK-induced iCCA cells. Finally, TAMs can suppress anti-tumor functions of T cells, an effect related to the hypoxia-inducible factor-1α (HIF-1α)-mediated up-regulation of arginase and iNOS in TAM, which deplete essential metabolic precursors such as L-arginine [20].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