CAF partners with Endometrial Tumor-Cells

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A tumor cell carrying characteristic genomic alteration(s) exists within its host's microenvironment. The tempero-spatial interaction of tumor cells with its microenvironment is the deterministic factor for tumor growth, progression, resistance to therapy, and its outcome in clinics. This manuscript presents a systemic review of the role of CAF in endometrial cancers. Here we present the functional characteristics of CAF in the context of endometrial cancers. We review **(1)** the characteristics of CAF, **(2)** their evolution from being anti-tumor to pro-tumor, **(3)** their involvement in regulating growth and several metastasis-associated phenotypes of tumor cells, **(4)** their participation in perturbing immune defense and evading immune surveillance, and **(5)** their role in mediating drug resistance via tumor-CAF cross-talk with particular reference to endometrial cancers. We interrogate the functional characteristics of CAF in the light of its dialogue with tumor cells and other components of TME towards developing a CAF-based strategy for precision therapy to supplement tumor-based therapy. The purpose of the review is to present a new vision and initiate a thought process which recognizes the importance of CAF in a tumor, thereby resulting in a novel approach to the design and management of the disease in endometrial cancers.

Keywords: tumor micro-environment ; cancer-associated fibroblasts ; metastasis-associated phenotypes ; immune-defense ; stromagenesis

1. Definition of CAF

Cancer-associated fibroblasts (CAFs) in solid tumors can be defined as a dynamically plastic host mesenchymal fibroblastic component of TME (tumor microenvironment) immediately surrounding the tumor cells. CAFs are a heterogeneous population of cells with extraordinarily numerous cells of origin and modes of activation [1][2][3][4][5][6][7][8][9]. CAFs are resident fibroblasts and other mesenchymal components of the host, which are transformed/activated by the tumor cell. **CAF presents a quadruple-negative status.** Based on lineage exclusion, CAFs are negative for (1) epithelial markers, (2) endothelial markers, (3) leukocyte markers, and (4) mutations found within cancer cells [9]. On the other hand, based on positivity for the mesenchymal marker(s) closely similar to normal fibroblasts, CAFs are positive for alpha-SMA (ACTA2), vimentin, FAP-1, FSP-1(fibroblast-specific protein-1; S100A4), CD90 (Thy-1), Tie-7, and PDGFR in different subsets.

2. CAF as an Evolving Component of Tumor Microenvironment (TME)

CAF is a component of TME. TME is the niche that provides metabolic support, immune surveillance, angiocrine, and inflammatory milieu to the tumor cells in a host's body. The TME is the deterministic and dynamic context of a solid tumor [10]. An evolving interaction is established via TME-tumor cross-talk. TME-tumor cross-talk and its evolution as a tumor progresses are critical events in regulating the tumor's fate, thus affecting the outcome of the management of a disease. Hence, tumorigenesis, when well supported by CAF, exhibits a higher probability of developing a progressive disease and drug resistance in response to treatment [9][11].

3. CAF & CAF Conundrum

CAF is a unique component of TME because it bears a strong element of ambiguity. CAFs present riddles; beginning with their definition, origin, markers, and functions in the context of tumorigenesis, tumor progression, and drug resistance. **First,** CAFs are characteristically different from the normal tissue-resident fibroblasts and yet retain their cardinal elongated fibroblastic-morphology, mesenchymal-markers, and major fibroblastic functions in a modified way [12]. **Secondly**, CAF cross-talks with all components of TME as well as tumor cells and thus bears a strong potential to become a target cell to contribute to the novel CAF-targeted treatment strategies [2][13][14][15]. Yet, CAFs are the least characterized, the least understood, and the most underutilized component of a tumor so far to aid the management of the disease. Although CAFs are the most abundantly present, easily cultured/modeled, and experimentally manipulated

critical elements of TME, their origin, markers, subpopulation, and functions remain largely inconclusive in many solid tumors. **Third,** within the TME of an advanced stage tumor, CAFs support tumor growth and metastasis of the tumor cells and co-evolve with the tumor progression and development of drug resistance. Yet CAF-directed management of solid tumors is minimal owing to the well-known multifaceted nature and function of CAFs [2][16]. This puzzling dichotomy is the single most characteristic nature of CAF, which makes them intriguing yet of limited applicability in the treatment of cancers.

4. CAF in Endometrial Cancers

Compared to solid tumors of other organ types, uterine endometrium presents an abundance of fibroblast-enriched stroma embedding the glandular epithelium. At a certain point of their oncogenic progression, endometrial cancer cells orchestrate the transformation of normal residential fibroblasts in the stroma into CAFs. In the TME of endometrial cancers, CAFs acquire cancer-specific characteristics in addition to their primarily fibroblastic background. Myofibroblast-rich cell populations constituting the tumor stroma associated with the host's immediate extra-tumoral cellular elements are designated as CAFs.

Modification of normal residential fibroblasts to activated/transdifferentiated myofibroblasts (a type of CAF) infiltrating carcinoma is irreversible as, once activated, CAFs preserve their pro-tumor property even in the absence of direct contact with tumor cells in vitro in many solid tumors [17][18][19]. Depending on the stages of neoplasia, tumor cells evolve due to their accumulation of many genetic changes, chromosomal aberrations, epigenetic changes, and they influence the co-evolution of all the components of TME, including CAFs [20]. The transformation from residential fibroblasts to CAF is a non-genomic alteration-mediated event in TME towards cancer progression. Thus CAFs play a critical role in ***"turning the table"*** in favor of tumor cells towards the progression and metastasis of the disease in patients, leading to a higher grade of malignancy and poor prognosis [21][22]. In the course of the real-time evolution of tumor cells, there occurs a rate-limiting conversion of CAFs in TME from pro-tumor state to anti-tumor state, "stromagenesis". In an established CAF-driven endometrial tumor, a cross-talk between CAF and tumor cells, the stromal milieu eventually acquires specific properties leading to a vicious circle of an incredibility complicated signaling choreograph among tumor cells, fibroblasts, pericytes, lymphocytes, endothelial cells, and tumor associated-macrophages. Thus, the mechanism of reverse conversion of CAFs from pro-tumor state to anti-tumor state will be essential to the construction of a disease management strategy based on targeting the reversal of "stromagenesis", stromal-switch, or normalization of stroma from pro-tumor to anti-tumor state.

Signaling pathways involved in the cross-talk between cancer cells and stromal CAF in gynecological malignancies, including endometrial cancers, may have therapeutic implications because of the role of the estrogen-mediated release of chemokines and cytokines [23]. CAF in the TME has a high therapeutic potential, offering many targeted and immunological therapies. In endometrial cancers, such clinical management of the disease based on targeting "stromagenesis" remains elusive at the present time due to the paucity of conclusive data to support the origin, subpopulation, heterogeneity, and definitive functions of CAF.

5. Characteristics of CAF-Tumor Cross-Talk

The functional relationship between CAF and tumor cells is one of the best examples of the TME-tumor cross talk. The cross-talk of CAF with tumor cells is the building block of the role of CAF. The CAF-tumor dialogue mediates all functions of CAF and thus controls the fate of tumor cells in real-time. Like other solid tumors, the cross-talk between CAF and tumor cells has been reported in gynecological malignancies, including uterine cancers, highlighting the high translational relevance of a CAF-based therapy in these organ-type cancers [23]. A genetically transformed tumor cell evolves its functions and relationships with stromal cells, including residential fibroblasts, which eventually get activated into CAFs. Cytokine secretion (CAF secretomes), cell-to-cell contacts (via co-stimulatory and co-inhibitor molecules), and exosomes are the language of this complex paracrine/juxtacrine intratumoral dialogue, which involves all potential phenotypes of a tumor cell, including tumor growth, angiogenesis, metabolic reprogramming, immune evasion, immune suppression, metastasis, and chemoresistance [8][24][25].

The CAF-tumor cross-talk is also bi-directional, dynamic, multi-nodal and involves: (1) transcriptional activation/repression of oncogenic factors, (2) the regulation of different oncogenic pathways at the miRNA or protein levels, and (3) the activation/suppression of cells belonging to the tumor compartment and other components of TME, such as the immune compartment or angiogenic compartment. The cross-talk between CAF and tumor cells can be stratified in two ways: (1) the modes of the cross-talk and (2) the tumor cell functions affected by the cross-talk. There can be several modes and mediators of the cross-talk in endometrial tumors, which can affect a number of cellular functions to control many phenotypes in the endometrial tumor cells.

The bi-directional cross-talk between CAF and tumor cells involves putative secretory signaling proteins such as TGF-beta 2, FGF2 (from CAF), and FGFR1 (from tumor cells). Interestingly PD-L1 expressed in CAF interacts with its cognate receptor, PD-1 expressed in the surrounding T-cells, one of the established cell-to-cell mechanisms of immune evasion. Although the cross-talk between CAFs and well-adapted tumor cells in other solid tumors has been identified historically [8][24][25][26][27], the functional relevance of the cross-talk and their influence on angiogenic and immune components of TME in endometrial cancers are yet to be fully characterized.

6. Language and Topic of Cross-Talk between CAF and Tumor Cells in Endometrial Cancers

Tumor cells are engaged to CAF via the paracrine/juxtacrine mode and vice versa [28]. The mode of signal transduction between CAF and tumor cells has been referred to as the “Language of Cross-Talk”, and how the cross-talk is involved or initiates different functions in the tumor cell has been referred to as the “Agenda of Cross-Talk”. The agendas of the cross-talk are: (1) growth, (2) proliferation, (3) metastasis-associated phenotypes, (4) metabolic reprogramming, and (5) immunological reprogramming. Most studies have been conducted on the effect of the CAF-tumor cell interaction in mediating the growth and proliferation of endometrial tumor cells. Interestingly, most of the CAF-mediated growth involved estrogen signaling via CAF secretome. In contrast, functions like metastasis-associated phenotypes, EMT, progression, and stemness were mediated via transcriptional regulation of genes, exosomes, and miRNAs. Although the studies on metabolic and immunologic reprogramming are limited in the current time in endometrial cancers, future work will identify the precise nature of this cross-talk. The details of CAF’s influence on different features of endometrial tumor cells have been presented in the review.

7. CAF Influencing Proliferation and Growth of Tumor Cells in Endometrial Cancers

Role of CAF in "Steroid-Driven Proliferation of Endometrial Tumor Cells" and "Non-Steroidal Proliferation of Endometrial Tumor Cells" are presented in the review.

8. CAF Influencing Metastasis-Associated Phenotypes of Tumor Cells in Endometrial Cancers

Role of CAF in "Matrix Organization & Stromal Architecture", "EMT", and "Migration, Invasion, and Metastatic Progression" are presented in the review.

9. CAF Influencing Immune-Defense of the Host and Immune-Surveillance of Tumor Cells by the Host in Endometrial Cancers

Role of CAF in "immune reprogramming" are presented in the review.

10. Epilogue

Here we presented the functional characteristics of CAF in the context of their origin. We reviewed the nature of CAF’s involvement in regulating growth and several phenotypes of tumor cells involving different oncogenic pathways. Thus, we interrogated the functional characteristics of CAF in the light of its dialogue with tumor cells and other components of TME towards developing a precision strategy of CAF-based therapy in solid tumors with particular reference to endometrial cancers.

A critical review of the subject at hand reveals two apparent facts in the context of CAF-tumor cell cross-talk in endometrial cancers. First, the literature for CAF is limited in endometrial cancers. Although a significant body of literature exists on the function of CAF and their deterministic role in shaping the disease progression in different solid tumors, like PDAC, breast cancers, lung cancers, colorectal cancers, and prostate cancers, the literature is limited for gynecological cancers, especially endometrial cancers. The insufficiency of data can be partly attributed to the impossibility of obtaining longitudinal sampling of the same lesion throughout the disease progression or during treatment to study a real-time conversion between two states of CAF, benevolent and malevolent, as well as their co-evolution with the tumor cells. The scope of repeat biopsy remains limited in the endometrial tumors, as so in many other cases of solid tumors. Second, considering the undeniable role of CAF in progression and drug resistance, the two most critical deterministic factors in the clinical management of a disease, it is puzzling why a CAF-based therapy has not evolved even in other solid tumors, where much more information exists. That brings us to the current state of the puzzle in CAF research. Literature from 1966–2021 favors the school of thought that CAFs are benevolent in suppressing the development of cancers [82,83]. It was demonstrated 50 years ago that normal fibroblasts inhibit the growth of polyoma virus-transformed cells [82]. Thus, it is argued that during the initial phase of tumorigenesis, the CAF, in its presumably inactivated state, may not be supportive to the growth of tumor cells. Conspicuously, CAF in its activated form is not the same in effecting tumor growth and progression, keeping the debate in favor or against CAF (good-CAF

versus bad-CAF) inconclusive and wide open even in the most studied solid tumors, wherein the contribution of stromal CAF is undeniable, including PDAC and breast cancers [3,4,14,22,84–86]. The malevolent transformation of CAF later is believed to be mediated through a number of tumor cell-initiated factors, immunogenic factors, and the physicochemical properties of stroma. In order to conclusively establish CAF as a target for a treatment, we need to establish definitive markers for good-CAF, which are mutually exclusive from the markers of bad-CAF, representing two distinct populations contextually connected to distinct clinical outcomes. To this end, a report by Mizutani et al. identified a Meflin-positive CAF in PDAC, which represented a cancer-restraining
population. Their data suggested that Meflin is a marker of cancer-restraining CAFs that suppress progression in PDAC [87,88]. Future studies may identify the presence of such markers in solid tumors, including endometrial cancers, and confirm their role in targeting such markers towards managing the disease.

From the interrogation of the current literature on the ambiguity of CAF, two relevant features surface. There are two characteristics of CAF that we know for certain. First is the heterogeneity in the CAF world, their origin, marker(s), and subpopulation(s). Second istheir undeniable role in initiating and the evolution of tumor cells that ultimately determine the disease's outcome. The first one restricts us from targeting CAF to achieve a successful CAF-based therapeutic strategy. The second one prohibits us from denying the scope of the CAF-based therapeutic approach towards managing the disease. The fact that a number of cross-talks exist between tumor cells and CAF in endometrial cancers proves the importance of the intra-tumoral CAF-tumor cells ecosystem. Furthermore, it explains why and how CAFs co-evolve with tumor cells during the process of metastatic progression of the disease and/or during the development of drug resistance following a clinical intervention, the two most critical determinants of the disease outcome. The only way out
of this puzzle is to acquire more knowledge about the heterogeneity and function of CAF, to establish a ***CAF-based stromal-switch***, and to address the dynamic contribution of CAF in the progression of cancer. As we begin an in-depth characterization of CAF and CAF's functional choreograph with endometrial tumor cells as well as other stromal cells within the uterine TME, studies will unearth novel therapeutic targets [23]. Future work will pave the way to stratify the approach to "normalize" the stromal switch by targeting CAF in endometrial cancers.

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