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Collective migration of cancer-associated fibroblasts is enhanced by overexpression of tight junction-associated proteins claudin-11 and occludin

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ABSTRACT

It has been suggested that cancer-associated fibroblasts (CAFs) positioned at the desmoplastic areas of various types of cancer are capable of executing a migratory program, characterized by accelerated motility and collective configuration. Since CAFs are reprogrammed derivatives of normal progenitors, including quiescent fibroblasts, we hypothesized that such migratory program could be context-dependent, thus being regulated by specific paracrine signals from the adjacent cancer population. Using the traditional scratch assay setup, we showed that only specific colon cancer cell lines (i.e. HT29) were able to induce collective CAF migration. By performing quantitative proteomics (SILAC), we identified a 2.7-fold increase of claudin-11, a member of the tight junction apparatus, in CAFs that exerted such collectivity in their migratory pattern. Further proteomic investigations of cancer cell line secretomes revealed a specific signature, involving TGF-β, as potential mediator of this effect. Normal colonic fibroblasts stimulated with TGF-β exerted myofibroblastic differentiation, occludin (OCLN) and claudin-11 (CLDN11) overexpression and cohort formation. Subsequently, inhibition of TGF-β attenuated all the previous effects. Immunohistochemistry of the universal tight junction marker occludin in a cohort of 30 colorectal adenocarcinoma patients defined a CAF subpopulation expressing tight junctions. Overall, these data suggest that cancer cells may induce CLDN11 overexpression and subsequent collective migration of peritumoral CAFs via TGF-β secretion.

Non-standard abbreviations: CAF, cancer-associated fibroblast; CALD, caldesmon; CLN11, claudin 11; CM, conditioned media; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; ICC, immunocytochemistry; IGP, invasive growth potential; IHC, immunohistochemistry; LAMA3, laminin a3; LAMB1, laminin b1; MEHP, mono-(2-ethylhexyl) phthalate; MWL, mean wound length; NMC/DSA, number of migrating cells per default squared area; OCLN, occludin; PLAU, urokinase-type plasminogen activator; SILAC, stable isotope labeling with amino acids in cell culture; TGF-β, transforming growth factor-beta.

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1. Introduction

One of the many crucial steps in cancer formation is the interaction between cancer and stromal cells at the invasive margins of the continuously expanding neoplasia. The aggressiveness of a particular carcinoma has been shown to be heavily dependent on the ability of the malignant cells to recruit the surrounding stromal cells. One particular subgroup of stromal cells, the cancer-associated fibroblasts (CAFs), is often found at the cancer invasive front, and has recently emerged as key player in this interaction. CAFs share a similar phenotype with myofibroblasts observed during wound healing and most of the times, they comprise reprogrammed variants of the quiescent fibroblast population (Hanahan and Weinberg, 2011; Kalluri and Zeisberg, 2006). Accumulation of peritumoral CAFs is often associated with increased deposition of extracellular matrix (ECM) components, such as collagens, fibrin, proteoglycans and glycosaminoglycans, a lesion also known as "desmoplasia" or "desmoplastic reaction" (Kunz-Schughart and Knuechel, 2002).

CAF populations found in different types or subtypes of cancer or even within the same cancer may share different gene and protein expression signatures. This heterogeneity explains the identification of a plethora of CAF subtypes, which, most of the times, present with diverse functional properties. There have been two possible explanations for this diversity.

First, CAFs may be derived from a wide variety of other progenitor cells beyond the pure fibroblastic subpopulation. For instance, bone marrow-derived circulating cells and myeloid precursors are able to localize and proliferate in the peritumoral stroma, specifically contributing to the myofibroblasts of the desmoplastic response, as well as angiogenesis (Direkze et al., 2004; Russo et al., 2006). Of note, the phenotypic switching of endothelial cells seems to also be context-dependent, as various cytokines present in their microenvironment, such as transforming growth factor-β (TGF-β), have been shown to induce a biological program termed endothelial-to-mesenchymal transition (Potenta et al., 2008). Indeed, a significant proportion (up to 40%) of CAFs may share endothelial markers such as PECAM/CD31, which implies that they originate from an endothelial subpopulation (Potenta et al., 2008). Remarkably, a special case of the epithelial-to-mesenchymal transition (EMT) program, which is deployed by cancer cells to efficiently assist their invasive/migratory behavior, may sequentially lead to the formation of CAFs, given that a permissive microenvironment exists. For instance, Petersen et al. (2001) showed that breast cancer cells may typically undergo an EMT event that transforms them into myoepithelial cells and a subsequent transdifferentiation event, which results in the generation of a non-malignant stroma consisting of CAFs (Petersen et al., 2001, 2003).

Second, recent evidence suggests that during cancer progression, tumor cells are able to alter the characteristics of the adjacent stroma and create a supportive microenvironment in a context-dependent way. In other words, cancer cells are dynamically altered (i.e. their genetic background is constantly changing), which allows them to secrete a diverse repertoire of growth factors and paracrine signaling molecules, which, in turn, are able to shape the tumor microenvironment in their favor and address any new challenges encountered in the metastatic cascade (Elenbaas and Weinberg, 2001; Serini and Gabbiani, 1999).

We have previously proposed a model of CAF-directed metastatic progression, according to which CAFs are able to migrate within the tumor microenvironment and the tumor compartment in a cohort configuration by developing a specific migratory, adhesive and paracrine signaling machinery (Karagiannis et al., 2013, 2012b). In that model, we additionally claimed that the recruitment of such machinery is causatively associated with increased metastatic behavior of the cancer cells Consistent with previous observations (Elenbaas and Weinberg, 2001; Scheel et al., 2011), here we conclude that the development of such machinery should be dependent on microenvironmental signals originating from the cancer cells, further closing this paracrine feedback loop. To demonstrate this, we hypothesized that stimulation of normal fibroblasts with secreted factors originating from cancer cell lines of different genetic backgrounds would allow us to observe their different migratory behavior.

2. Experimental procedures

2.1. Reagents

The recombinant human TGF-β1, the pan-TGF-β neutralizing antibody and the tight junction inhibitor mono-(2-ethylhexyl) phthalate (MEHP) were purchased from Sigma–Aldrich. The active occludin-disrupting peptide LYHY and the corresponding control peptide LYQY were purchased from CanPeptide.

2.2. Cell culture

The human colon cancer cell lines HT29, SW480 and SW620, and the normal colonic fibroblast cell line 18Co, were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and maintained in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% Fetal Bovine Serum (FBS), and 1% penicillin/streptomycin, in a humidified atmosphere of 5% CO_2, at 37 °C. All experiments were conducted before passage #8 from the initiation of all cultures. For stimulations, conditioned media (CM) from 18Co, HT29, SW480 and SW620 cells were generated in serum-free (SF) conditions. Briefly, the cells were seeded at 50% confluence in T175 flasks, in DMEM with 10% FBS for 12 h to allow for adherence and proliferation. Then, the flasks were washed with phosphate buffered saline (PBS) twice and SF medium (chemically-defined Chinese hamster ovary; CDCHO) was added in the cultures for 2 days. Then, all the media were collected, centrifuged at 1500 rpm for 5 min to remove dead cells and were concentrated 4 times, using a 5 kDa membrane cut-off. Concentrated media were rediluted 4 times, in fresh SF medium, to enrich for nutrients, and subsequently filter-sterilized through a 0.22 μm membrane cut-off. For the SILAC experiment, labeled SF stimulation media were generated to not disturb the metabolic incorporation of the heavy and light amino acid isotopes.

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