Investigation of FAK signaling in mesenchymal stem cells-induced Epithelial-to-Mesenchymal Transition of colorectal cancer cells

França, L. C. [1,2]; **Barcellos-de-Souza, P.** [1]; **Rocha, M. R.** [1]; **Du Rocher, B.** [3]; **Abdelhay, E. S.** [4]; **Morgado-Díaz, J. A.** [1]

1 Grupo de Estrutura e Dinâmica Celular, Programa de Oncobiologia Celular e Molecular, Centro de Pesquisas – INCa; 2 Faculdade de Farmácia, Universidade Federal do Rio de Janeiro; 3 Laboratório de Pesquisa sobre o Timo, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz; 4 CEMO, INCA.

ABSTRACT

The tumor microenvironment is functionally important for tumor development and progression. It comprises many cell types including cancer-associated fibroblasts (CAF) and mesenchymal stem cells (MSC). MSCs are multipotent cells derived from the bone marrow that can differentiate into osteoblasts, chondrocytes, adipocytes and other cells of mesenchymal origin. MSCs are recruited by cancer cells to enhance their survival, growth, invasion and dissemination, however, it is controversial whether MSCs promote or inhibit tumor progression. It has been reported that under certain circumstances MSC can transdifferentiate into active players in tumor progression, such as CAF. Recently it has been shown that crosstalk between tumor cells and MSCs increases metastatic potential and promotes epithelial-to-mesenchymal transition (EMT). EMT is a cellular process during which epithelial cells lose their polarized organization and cell-cell junctions and acquire mesenchymal characteristics. This process is thought to enhance motility, invasion, and apoptosis resistance. As the role of MSCs in the tumor microenvironment remain unclear, this study aims to characterize the relationship between MSCs and signaling pathways triggered during EMT in colorectal cancer cells (CRC).

Here, we found that bone marrow-derived MSCs are recruited and induced to acquire a CAF-like phenotype with high expression of alpha smooth muscle actin (α-SMA) in the presence of HCT-116 conditioned medium. Once activated, MSCs promoted EMT in HCT-116 cells as indicated by downregulation of the epithelial marker E-cadherin, upregulation of mesenchymal markers Vimentin and α–SMA, and acquisition of mesenchymal morphology. Additionally, HCT-116 cells displayed increased invasiveness when cultivated with MSCs conditioned medium. Then, we started to evaluate the signaling pathways likely to be responsible for MSC-mediated EMT. Initially, we focused on the Focal-adhesion kinase (FAK) signaling that is involved in cellular events, such as proliferation, migration, invasion and tumor angiogenesis. Therefore, we investigated the kinetics of FAK phosphorylation at Tyr-397 and Tyr-861 (previously associated with enhanced migration and metastatic potential in prostate cancer cells) in response to TGF-β, an important inducer of EMT. In the first 15 min, there was an initial FAK activation by phosphorylation at both sites before Src activation. After 24h, the HT-29 cells displayed an increased expression of Yes protein, a Src family kinase. Moreover, both pFAK Y397 and pFAK Y861 were upregulated in HT-29 cells undergoing EMT, after 48h stimulation. We also demonstrated by immunofluorescence that upon TGF-β treatment pFAK Y397 was mainly localized in the cytoplasm, whereas pFAK Y861 was found in the focal adhesions, demonstrating colocalization with the focal adhesion marker Vinculin in HT-29 cells. To assess whether FAK activation was also involved in the MSC-mediated EMT, we treated HCT-116 cells during 48h with MSCs conditioned medium and we verified an increased pFAK Y397, pFAK Y861 and Yes expression. Collectively, these data suggest that Yes/FAK signaling axis might have a potential role in the MSC-mediated EMT, inducing a more malignant phenotype in CRC cells.

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RESULTS

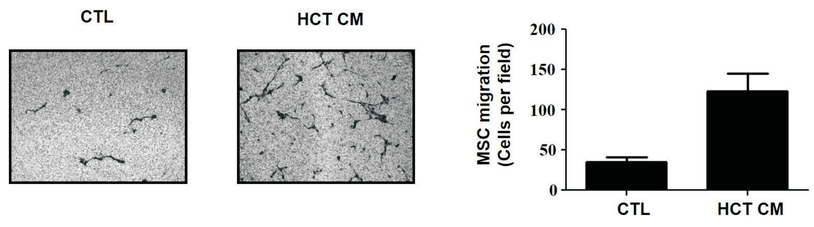

Figure 1. Bone marrow-derived MSCs are recruited to HCT-116 conditioned medium. Cells were seeded in the upper chamber of transwell inserts and were allowed to migrate toward HCT-116 conditioned medium (HCT CM) or control medium (CTL) for 24h. Results are shown mean ± SD of triplicates in the same experiment.

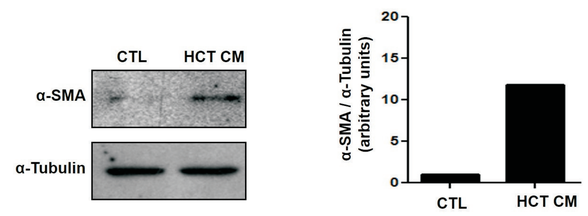

Figure 2. HCT-116 conditioned medium induces a CAF-like phenotype in MSCs. α-SMA expression was analyzed by Western blotting after MSCs treatment over 24h with HCT-116 conditioned medium (HCT CM) and the densitometric analysis were performed. The untreated cells were used as control.

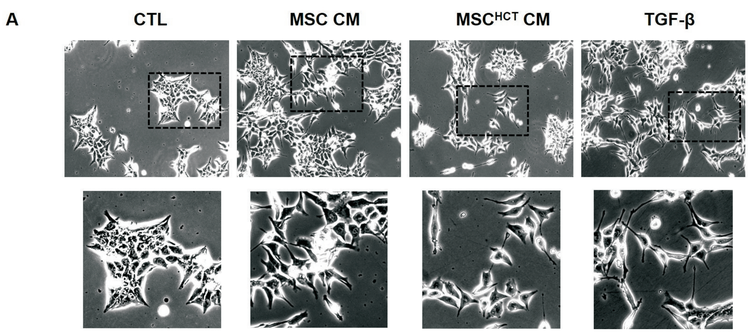

Figure 3. Activated MSCs induce EMT in HCT-116 cells. (A) Representative images of morphological changes in HCT-116 cells after 48h treatment with MSC conditioned medium (MSC CM), MSC activated by HCT-116 conditioned medium (MSCHCT CM), or TGF-β (10 ng/mL). Image magnification: 100x and 200x (insert). (B) Protein levels of epithelial marker E-cadherin, and the mesenchymal markers Vimentin and α-SMA were assessed by Western blotting. GADPH protein expression was used as loading control.

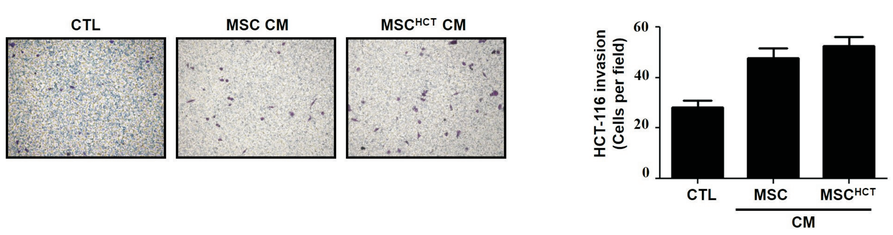

Figure 4. Activated MSCs promote invasiveness of HCT-116 cells. The invasive capacity of HCT-116 was assessed by Matrigel invasion assay after 24h treatment with control medium, MSC conditioned medium (MSC CM) or MSC activated by HCT-116 conditioned medium (MSCHCT CM). Results are shown mean ± SD of triplicates in the same experiment.

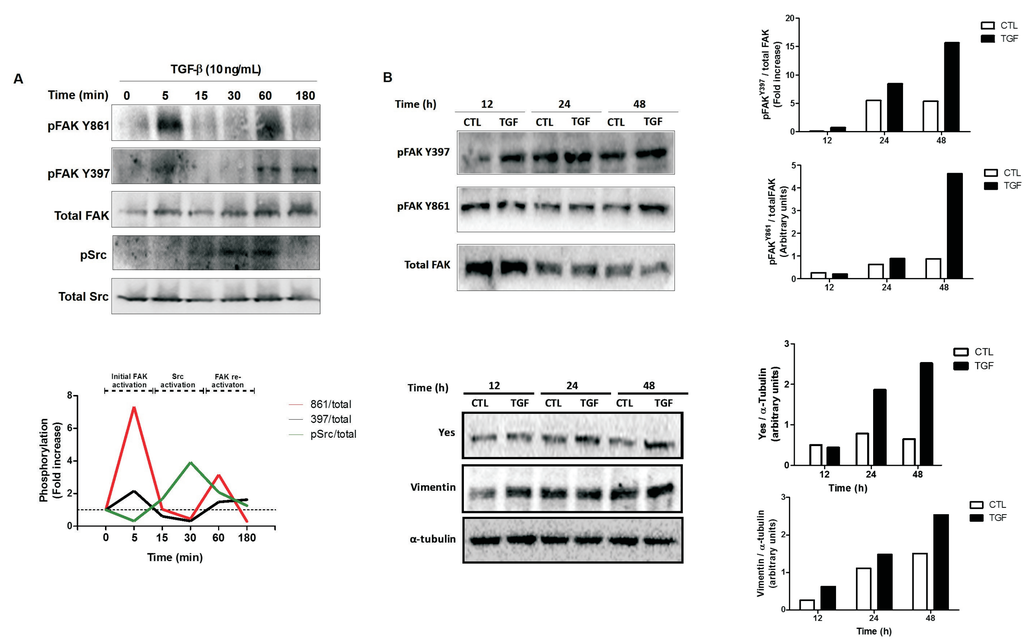

Figure 5. Kinetics analysis of FAK phosphorylation in HT-29 cells upon TGF-β treatment. (A) Representative images of Western blotting and densitometric analysis of FAK and Src expression in HT-29 cells after TGF-β treatment (10 ng/mL), 0-180 min. (B) Representative images of Western blotting and densitometric analysis of FAK, Yes and Vimentin expression after TGF-β treatment (10 ng/mL), 12-48h. α-tubulin protein expression was used as loading control.

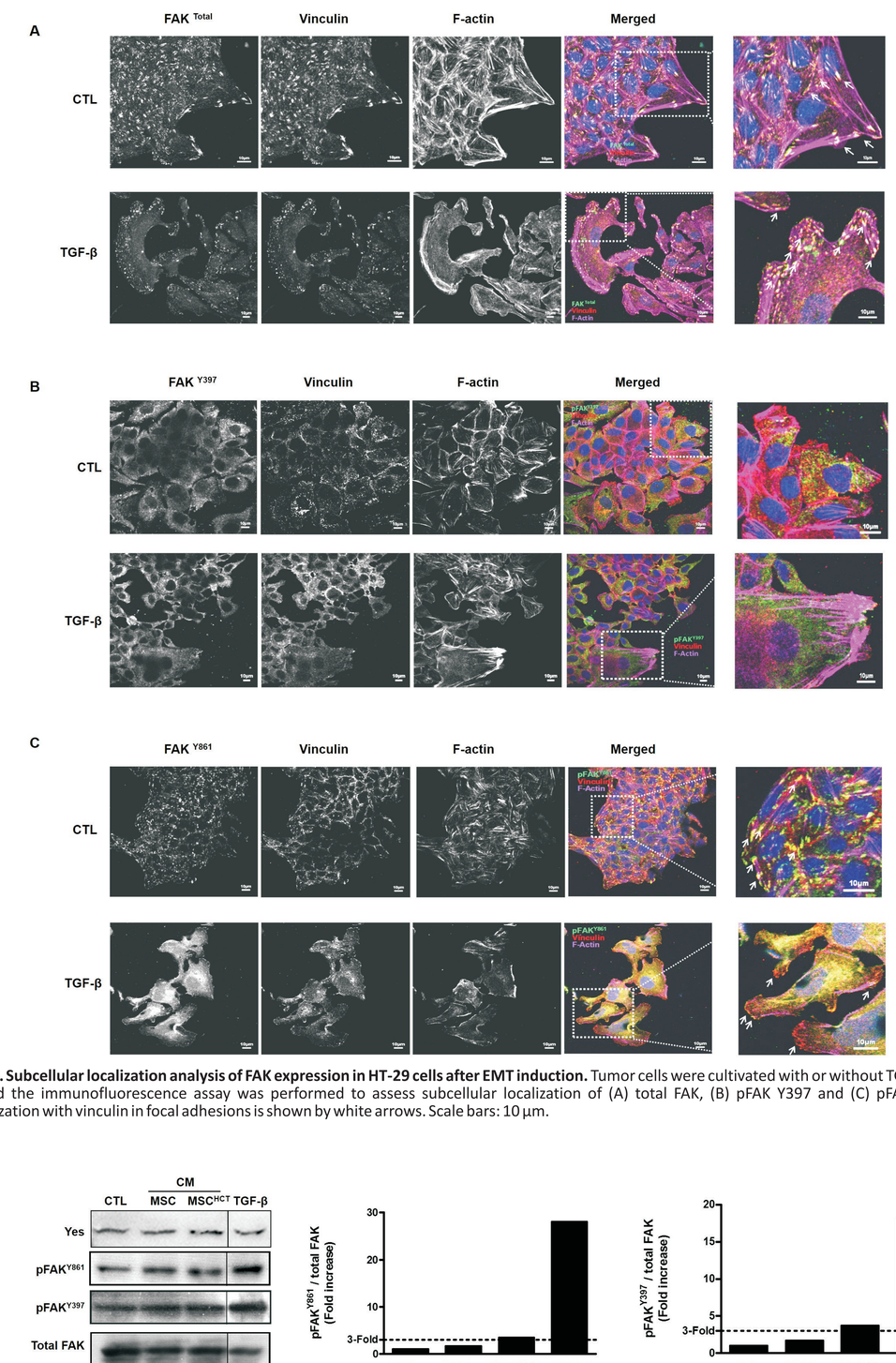

Figure 6. Subcellular localization analysis of FAK expression in HT-29 cells after EMT induction. Tumor cells were cultivated with or without TGF-β (10 ng/mL) over 48h and the immunofluorescence assay was performed to assess subcellular localization of (A) total FAK, (B) pFAK Y397 and (C) pFAK Y861. pFAK Y861 colocalization with vinculin in focal adhesions is shown by white arrows. Scale bars: 10 µm.

Figure 7. Activated MSCs promote Yes, pFAK Y861 and pFAK Y397 upregulation in HCT-116. Cells were treated with MSC CM, MSCHCT CM or TGF-β (10ng/mL) over 48h. The protein levels were assessed by western blotting and densitometric analysis were performed.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA