Snail1-expressing fibroblasts as a source of paracrine signals in colon cancer tumors

Alberto Herrera1, Mercedes Herrera1, Félix Bonilla2, Antonio García de Herreros3, Cristina Peña1

1Grupo de investigación en “Señalización celular en cáncer”, Hospital Universitario Puerta de Hierro de Majadahonda, Majadahonda, Madrid, 28222, Spain
2Centro de Estudios Biosanitarios, Madrid, 28029, Spain
3Programa de Recerca en Càncer, IMIM-Hospital del Mar, Barcelona, 08003, Spain

Correspondence: Cristina Peña Maroto
E-mail: cpena@idiphim.org
Received: November 12, 2014
Published online: December 25, 2014

RESEARCH HIGHLIGHT

Snail1 protein (SNAI1) is a transcription factor for the Epithelial-Mesenchymal Transition (EMT), which is characterized by the loss of epithelial characteristics and the acquisition of mesenchymal traits such as greater motility [1]. During embryo development Snail1 expression and EMT are observed at different stages, and they are required for numerous processes such as gastrulation and neural crest formation [2]. In cancer cells, Snail1 represses E-cadherin gene (CDH1) and induces EMT, indicating its fundamental role in cancer progression [3,4]. Snail1 expression together with different EMT characteristics are detected in different types of cancer as breast, prostate, lung, ovarian, melanoma, colon or esophageal cancers [5–14]. In most cases, Snail1 correlates inversely with CDH1 expression [6,15–17]. Indeed, overexpression of Snail1 or reduced CDH1 expression is associated with higher tumor grade, nodal metastasis and its associated with lower survival times in patients with different types of cancer [3,4].

The tumor microenvironment has attracted attention as a therapeutic area because it plays an essential role in stimulation of tumorigenesis, controls tumor drug-uptake and sensitivity, and has an impact on prognosis. Moreover, there is growing evidence that the microenvironment also stimulates EMT, thereby enhancing the invasive properties of cancer [18]. Fibroblasts are a major component of the stroma as well as one of the most active cell types. They contribute to tumor biology in several ways, such as extracellular matrix remodeling, the release of soluble factors, and the regulation of tumor cell motility, metabolism or implantation [19–23]. Fibroblasts of the tumor stroma have received various names: tumor-associated fibroblasts, myofibroblasts or carcinoma-associated fibroblasts (CAFs), as we refer to them here [24]. CAFs comprise a heterogeneous population of cells derived from several sources [18]. The heterogeneity of CAFs may reflect the variety of their involvement in cancer progression. In an earlier study, we reported that the degree of activation...
of CAFs, as measured by classical activated fibroblast markers, predicts outcome in colon cancer patients [25]. Moreover, a specific prognostic gene expression signature was derived from the functional heterogeneity of CAFs [26]. Exploring the heterogeneity of CAFs may help us to understand epithelial-stromal interactions, which could ultimately inform about cancer pathogenesis, natural history and future therapeutics.

We have previously reported that, in colon tumors, Snail1 expression is observed in cells with a fibroblast-like phenotype into the tumor stroma [11]. Furthermore, colon cancer patients with Snail1 expression in tumor stroma showed poor outcomes [11]. In parallel, we described the paracrine effects on human colorectal cells derived from Snail expression in neighboring tumor cells, showing down-regulation of CDH1 and VDR expression when epithelial cells were co-cultured with Snail1 over-expressing colon cancer cells [17]. However, the molecular mechanisms underlying the association between Snail1 expression in the stroma and colon cancer patients’ survival, or the derived paracrine mechanism generated by Snail1-expressing cells are not yet clear. Although many studies have focused on the effects of Snail1 expression in tumor cells, few have analyzed Snail1 expression in fibroblasts isolated from tumor stroma. Accordingly, in a recent study [27], we described colon cancer cell migration and proliferation enhancement driven by paracrine-derived effects of Snail1-expressing fibroblasts involving novel cytokine profile.

In the work by Herrera et al. [27], primary cultures of Cancer-Associated Fibroblasts (CAFs) from colon cancer patients and fibroblasts from normal colon mucosa (NFs) were established to determine variation in Snail1 expression. Interestingly, CAFs showed higher Snail1 expression levels than fibroblasts from normal colon mucosa indicating that Snail1 can be considered a CAF marker. Furthermore, an association of Snail1 expression and other markers of CAFs such as α-SMA and FAP, was observed in normal and tumor samples of colon cancer patients, either by RNA analysis or by co-staining.

We have also followed alternative approaches to examine the pro-tumorigenic effects of Snail1 expression in fibroblasts on colon cancer cells, including in vitro migration models (with a set of colon cancer and fibroblast cells), primary CAFs and an in-vivo co-xenograft model. The data indicated that Snail1 expression in fibroblasts increases the tumorigenic capacity of co-cultured colon cancer cells to stimulate both cell migration and proliferation [27]. The enhanced migration response of target cells was not restricted to their co-culture with Snail1-expressing fibroblasts, since it was also observed in the presence of Snail1-transfected epithelial cells. However, the increase in colon tumor cell migration was stronger when Snail1 was expressed by fibroblasts. These results are consistent with the decrease in E-Cadherin mRNA expression observed in colon cells co-cultured with Snail1-expressing cells [17]. This E-cadherin down-modulation, together with the increase in migration of colon cells co-cultured with Snail1-expressing fibroblasts, might indicates that fibroblasts induce EMT or partial EMT in colon epithelial cells.

On the other hand, the association between retarded cell proliferation and EMT has been described, since cell division is impaired in Snail1-expressing epithelial cells [28–30]. However, association with Snail1 and increase in proliferation has also been reported in other studies. Thus, down-regulation of E-cadherin together with increased proliferation are associated with Snail1 expression under hair-bud formation [31]. Nevertheless, in the studies by Nieto, Cano and co-workers Snail1 was over-expressed in epithelial cells, while we analyzed Snail1 in fibroblasts; the effect of Snail1 might not be the same in a different cellular background. Accordingly, Snail1 expression in fibroblasts does not retard cell growth [32,33].

A series of studies confirmed that Snail1 overexpression in epithelial cells enhances migration of different cell types, through the release of pro-inflammatory cytokines [34–36]. Although further studies are required to identify the fibroblast-derived mediators of Snail1-dependent stimulatory effects in vitro, these results indicate that, in fibroblasts, Snail1 expression induces the reprogramming of the cytokinome [17]. Therefore, Snail1 enhances MCP-3 expression in CAFs or fibroblastic cell lines, and the expression of both genes is associated in human colon cancer samples. Therefore, MCP-3 is a possible mediator of Snail1-dependent paracrine effects on cancer cell migration and proliferation [17]. Other studies support the relevance of this cytokine: for instance, metastatic colorectal cancer cell lines were characterized by higher expression of MCP-3/4 as compared to primary tumor cell lines [37]. MCP-3 has also been related with matrix invasion, lymph node metastasis and tumor node metastasis in gastric cancer [38]. In a co-culture of oral squamous carcinoma cells and CAFs, MCP-3 was up-regulated and promoted the invasion and migration of tumor cells [39]. Remarkably, shRNAs directed against MCP-3 suppressed tumorigenicity following co-injection of tumor cells and fibroblasts, since tumor cell proliferation is reduced to the same levels observed in derived tumors without fibroblasts co-injection [40].

Briefly, the study highlights the importance of Snail1 expression in CAFs and fibroblasts, enhancing the tumorigenic capacity of colorectal cancer cells, possibly
attributable to a change in the profile of secreted cytokines [27]. Although its role in epithelial cells has received more attention, various studies have analyzed the effects of Snail1 expression in the regulation of other cell types in the tumor microenvironment, such as fibroblasts or mesenchymal stem cells. For instance, Snail1 expression is required for the maintenance of mesenchymal stem cells, preventing differentiation to adipocytes or osteoblasts, and it controls the tumorigenic properties of these cells in the generation of sarcomas [32, 41]. Moreover, Snail1 is also required for the control of extracellular matrix architecture by fibroblasts, and to guide epithelial tumor migration [42]. Finally, tissue-invasive potential and angiogenesis induction is not detected in Snail1-depleted fibroblasts [33].

All these findings emphasize the need to elucidate the cross-talk between fibroblast and cancer cells and to identify the cytokines involved. Moreover, inhibition of fibroblast-epithelial interactions represents a strategy for interference with cancer progression, encouraging the search for new therapies which could offer a synergistic effect with the current systemic therapies.

Conflict of interest

All authors declare that there is no conflict of interest.

Acknowledgements

R. Rycroft helped with the English text. This research was supported by PI12/02037, the Fundación Científica AECC, SAF2010-20750, S2010/BMD-2344, RTICC-RD12/0036/0041, and by the Fundación Banco Santander. Antonio García de Herreros’ laboratory was supported by RTICC-RD12/0036/0005 and SAF SAF2013-48849-C2-1-R. Cristina Peña is a recipient of Miguel Servet Contract from the Instituto de Salud Carlos III.

References


