MOLECULAR ONCOLOGY XXX (2014) 1–16

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/molonc

Differences between CAFs and their paired NCF from adjacent colonic mucosa reveal functional heterogeneity of CAFs, providing prognostic information

Mireia Berdiel-Acer[a], *Rebeca Sanz-Pamplona*[b], *Alexandre Calon*[c], *Daniel Cuadras*[b], *Antoni Berenguer*[b], *Xavier Sanjuan*[d], *Maria José Paules*[d], *Ramon Salazar*[e], *Victor Moreno*[b], *Eduard Batlle*[c], *Alberto Villanueva*[a], *David G. Molleví*[a,*]

[a]*Translational Research Laboratory, Catalan Institute of Oncology, IDIBELL, L'Hospitalet de Llobregat, 08908 Catalonia, Spain*
[b]*Biomarkers and Susceptibility Unit, Cancer Prevention and Monitoring Programme, Catalan Institute of Oncology, IDIBELL, L'Hospitalet de Llobregat, Catalonia, Spain*
[c]*Oncology Programme, Institute for Research in Biomedicine IRB, Barcelona, Catalonia, Spain*
[d]*Pathology Department, Hospital Universitari de Bellvitge, IDIBELL, L'Hospitalet de Llobregat, Catalonia, Spain*
[e]*Medical Oncology Department, Catalan Institute of Oncology, IDIBELL, L'Hospitalet de Llobregat, Catalonia, Spain*

ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form
14 April 2014
Accepted 15 April 2014
Available online ■

Keywords:
Carcinoma-associated fibroblasts
Microenvironment
Stroma
Biomarker
Colorectal

ABSTRACT

Little is known about the difference in gene expression between carcinoma-associated fibroblasts (CAFs) and paired normal colonic fibroblasts (NCFs) in colorectal cancer. Paired CAFs and NCFs were isolated from eight primary human colorectal carcinoma specimens. In culture conditions, soluble factors secreted by CAFs in the conditioned media increased clonogenicity and migration of epithelial cancer cells lines to a greater extent than did NCF. *In vivo*, CAFs were more competent as tumour growth enhancers than paired NCFs when co-inoculated with colorectal cell lines. Gene expression analysis of microarrays of CAF and paired NCF populations enabled us to identify 108 deregulated genes (38 upregulated and 70 downregulated genes). Most of those genes are fibroblast-specific. This has been validated *in silico* in dataset GSE39396 and by qPCR in selected genes. GSEA analysis revealed a differential transcriptomic profile of CAFs, mainly involving the Wnt signallingsignalling pathway, focal adhesion and cell cycle. Both deregulated genes and biological processes involved depicted a considerable degree of overlap with deregulated genes reported in breast, lung, oesophagus and prostate CAFs. These observations suggest that similar transcriptomic programs may be active in the transition from normal fibroblast in adjacent tissues to CAFs, independently of their anatomic demarcation. Additionally NCF already depicted an activated pattern associated with inflammation.
The deregulated genes signature score seemed to correlate with CAF tumour promoter abilities *in vitro*, suggesting a high degree of heterogeneity between CAFs, and it has also prognostic value in two independent datasets.

Abbreviations: DEG, differentially expressed gene; CAF, carcinoma-associated fibroblast; NCF, normal colonic fibroblasts; ECM, extracellular matrix; CM, conditioned medium.

* *Corresponding author*. Tel.: +34 93 2607370; fax: +34 93 2607466.
E-mail addresses: dgmollevi@iconcologia.net, davidg.mollevi@gmail.com (D.G. Molleví).

1574-7891/$ – see front matter © 2014 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.molonc.2014.04.006

Please cite this article in press as: Berdiel-Acer, M., et al., Differences between CAFs and their paired NCF from adjacent colonic mucosa reveal functional heterogeneity of CAFs, providing prognostic information, Molecular Oncology (2014), http://dx.doi.org/10.1016/j.molonc.2014.04.006

ARTICLE IN PRESS

Further characterization of the roles these biomarkers play in cancer will reveal how CAFs provide cancer cells with a suitable microenvironment and may help in the development of new therapeutic targets for cancer treatment.

© 2014 Federation of European Biochemical Societies.
Published by Elsevier B.V. All rights reserved.

1. Introduction

The tumour microenvironment is a place where tumour cells co-exist and co-evolve with "non-tumoral" cells such as adipocytes, fibroblasts, endothelial and immune cells, all of which are embedded in an extracellular matrix (ECM). When changes occur in this compartment, the altered stroma can influence cancer development and progression (Bissell et al., 1982; Bissell and Hines, 2011). Carcinoma-associated fibroblasts (CAFs), the main constituents of tumour stroma, actively drive tumourigenesis and cancer progression (Elenbaas and Weinberg, 2001; Hwang et al., 2008; Olumi et al., 1999; Orimo et al., 2005; Zhang et al., 2009). They participate throughout tumour development by establishing cell–cell interactions with tumour cells or through secretion of cytokines, chemokines and growth factors. CAFs are most often denoted by the expression of α-smooth muscle actin (αSMA), but other markers such as vimentin and fibroblast-activating protein (FAPα) are also used to identify them. Nevertheless, these are also markers of myofibroblasts or activated fibroblasts, and not properly and exclusively of CAFs. An exclusive marker for CAFs that can clearly distinguish them from normal fibroblasts from adjacent mucosa or other closely related cell types is yet to be identified. Chang et al. (2002) reported that transcriptional patterns displayed by fibroblasts from different anatomic sites were distinct and characteristic, and suggested that fibroblasts from different organs could be considered as distinct differentiated cell types. However, in a subsequent study, the same group defined a common transcriptomic profile in fibroblasts stimulated with serum and with a pattern similar to that observed in a wound healing process and associated with tumour progression (Chang et al., 2004). It is known that the involvement of CAFs in solid tumours, in which desmoplasia is a characteristic feature, is of huge importance. Gene expression profiles and their association with cancer aggressiveness, has been reported in several tumours types (Peng et al., 2013). The current work is focused on colorectal cancer, in which, to our knowledge, the genetic and molecular profile of CAFs has been less thoroughly explored. The purpose of this study is to identify, for the first time in paired samples, genes that are differentially expressed in two fibroblast populations derived from the same colorectal cancer (CRC) patients (NCFs and their paired CAFs from primary tumour) and to examine their associations with phenotypic differences. Using microarray technology, we identified 109 deregulated probes (corresponding to 108 genes) differentially expressed between the two populations and functionally involved in cancer progression. Knowledge of alterations in the stroma surrounding a tumour might provide tools and biomarkers of use in designing new ways to attack tumour-supportive CAFs and could add valuable information for future and prognostic treatments.

2. Materials and methods

2.1. *Culture of primary fibroblasts and preparation of conditioned medium*

Fresh surgical specimens from colorectal cancer patients were obtained with the approval of the Ethics Committee of the Hospital Universitari de Bellvitge (IDIBELL). Tissue samples from morphologically normal colonic mucosa (at least 5 cm from the surgical margin), and from colorectal primary tumour were minced and incubated with collagenase and dispase for 2 h at 37 °C. Cells were resuspended and plated with Dulbecco's modified Eagle's medium-F12 (DMEM F12, Gibco) containing 10% fetal bovine serum (FBS) and penicillin/streptomycin antibiotics. Primary fibroblast cultures were established and routinely maintained at 37 °C in a humidified atmosphere containing 5% CO_2. After a maximum of 5 passages, RNA and protein were obtained to check for fibroblast purity. 10^6 fibroblasts were incubated for 48 h in 10-cm diameter dishes in DMEM-F12 with or without FBS. Conditioned medium was collected, centrifuged for 5 min at 3000 rpm to remove cell debris, sterile-filtered through 0.22-μm filter units (Millex® GS, Millipore) and stored at −80 °C until use.

2.2. In vitro *cellular assays*

Migration of cancer cells and CAFs was measured by wound healing assay. Cells were seeded in 6-cm diameter plates and cultured until confluent. The cell monolayer was scratched with a yellow 200-μl pipette tip to create a wound. After several PBS (phosphate-buffered saline 1×) washes to remove floating cells, in an epithelial cell migration assay, conditioned medium from NCF or CAF was added. Pictures were taken at different times. Distances between cell margins were measured with Leica software (Wetzal, Germany) on three occasions and each assay was performed in duplicate. Clonogenic capacity was assessed by cloning assay. We plated 100 cells for each epithelial colon cancer cell line (DLD1, SW620, SW480 and SW1116) in 12-well plates and incubated them for 9 days in DMEM F12 10% (control) or the appropriate conditioned medium. The number of colonies was counted after crystal violet staining. A WST-1 cell proliferation assay was conducted in CAFs alone and in DLD1 cells stimulated with CAF conditioned medium (CM) (24 h without FBS being collected, as mentioned above). Briefly, 1000 cells were seeded in a 96-well plate and cultured at several times (0, 24, 72 and

Please cite this article in press as: Berdiel-Acer, M., et al., Differences between CAFs and their paired NCF from adjacent colonic mucosa reveal functional heterogeneity of CAFs, providing prognostic information, Molecular Oncology (2014), http://dx.doi.org/10.1016/j.molonc.2014.04.006

Download English Version:

https://daneshyari.com/en/article/10914851

Download Persian Version:

https://daneshyari.com/article/10914851

Daneshyari.com