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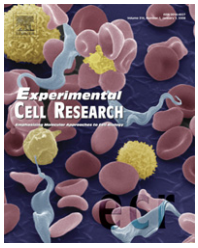

Research Article

Human mesenchymal stem cell transformation is associated with a mesenchymal–epithelial transition

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ABSTRACT

Carcinomas are widely thought to derive from epithelial cells with malignant progression often associated with an epithelial–mesenchymal transition (EMT). We have characterized tumors generated by spontaneously transformed human mesenchymal cells (TMC) previously obtained in our laboratory. Immunohistopathological analyses identified these tumors as poorly differentiated carcinomas, suggesting that a mesenchymal–epithelial transition (MET) was involved in the generation of TMC. This was corroborated by microarray and protein expression analysis that showed that almost all mesenchymal-related genes were severely repressed in these TMC. Interestingly, TMC also expressed embryonic antigens and were able to integrate into developing blastocysts with no signs of tumor formation, suggesting a dedifferentiation process was associated with the mesenchymal stem cell (MSC) transformation. These findings support the hypothesis that some carcinomas are derived from mesenchymal rather than from epithelial precursors.

Introduction

A hierarchical model is associated with stem cell differentiation. In this model, totipotent stem cells differentiate to multipotent stem cells, which in turn can give rise to pluripotent stem cells, which generate committed precursors that differentiate terminally to mature cells [1]. Some groups have nonetheless proposed modifications to this strict hierarchy, with processes such as cell dedifferentiation, by which a cell could generate a precursor with greater potential than the cell from which it derives [2], or cell transdifferentiation, that allows cell lineage switch [3].

The epithelial–mesenchymal transition (EMT) is a physiological mechanism present during development, and is also encountered in several pathological situations such as renal interstitial fibrosis, endometrial adhesion, and cancer metastasis [4]. Some primary carcinoma cells undergo EMT; these mesenchymal-like cells then secrete proteases that promote degradation of extracellular matrix and acquire movement capacity, allowing escape from the primary tumor, migration

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Abbreviations: MSC, Mesenchymal stem cells; TMC, Transformed mesenchymal cells; MET, Mesenchymal–epithelial transition; EMT, Epithelial–mesenchymal transition

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to the bloodstream or lymph, and extravasation to generate metastases [5–7]. These metastases, nonetheless, have an epithelial phenotype, suggesting reversibility of EMT and indicative of a mesenchymal–epithelial transition (MET). MET also takes place during normal development, in processes such as somitogenesis, kidney development, and coelomic cavity formation [8–10], but to date has not been associated with tumors derived from mesenchymal cells.

Cancer stem cells (CSC) have been reported for some tumor types, including breast and lung cancer, leukemia and glioblastoma [11]. Adult stem cells and CSC share several features including self-renewal ability, asymmetric division and differentiation potential [12]. Altered stem cells are thought to give rise to CSC in certain cases [13], and we recently described an *in vitro* model of spontaneous CSC generation from mesenchymal stem cells [14]. A recent report has suggested that both EMT and MET may be involved in the malignant progression of epithelial tumors. In this model, CSC in the primary epithelial tumor, undergo EMT to give rise to migrating CSC, which reconvert to a stationary epithelial-like CSC after an MET at the site of metastasis [15].

Here we show that spontaneously transformed MSC when injected into mice gave rise to primitive carcinomas with an epithelioid phenotype. These tumor cells down-regulated almost all mesenchymal-related transcripts and repressed vimentin, a mesenchymal protein, whereas they expressed epithelial antigens such as cytokeratins. TMC cells were able to integrate into developing blastocysts and contributed to placental tissue. These results show that spontaneous MSC transformation involves a mesenchymal–epithelial transition associated with a dedifferentiation process.

Materials and methods

Isolation of human mesenchymal stem cell and cell culture. Human mesenchymal stem cell isolation and culture was described previously [14]. Briefly, samples of adipose tissue were minced and digested with 1 mg collagenase P (Roche)/0.5 g sample/ml DMEM (37 °C, 1 h). Samples were clarified by sedimentation, the resulting cell suspension filtered through a 40-mm^2 nylon filter (Becton Dickinson), plated onto tissue culture plastic (10^3 cells/cm^2), allowed to adhere during 48 h and washed twice with phosphate buffer. Adipose tissue-derived MSC from C57BL/6Tg15(act-EGFP)Osb1 strain mice were isolated using the same method. Adherent cells were cultured at 37 °C and 5% CO_2 in MSC medium (DMEM plus 10% FCS, 2 mM glutamine, 50 μg/ml gentamycin). Transformed mesenchymal cells (TMC) were cultured in the same conditions. Human samples were obtained according to Spanish and European guidelines.

In vivo tumorigenesis

Eight-week-old male BALB/cJHanHsd-Prkdc^{scid} (Harlan) mice were infused subcutaneously with 10^7 cells of EGFP-TMC. Mice were killed when tumors reached approximately 2 cm^3. Tumors were removed and all organs were analyzed under UV light to detect EGFP-positive cells or fixed for immunohistochemistry analysis. Mice were maintained under high standards conditions in accordance with FELASA (Federation of European Laboratory Animal Science Associations) procedures. All the experiments were conducted according to the Spanish and European regulation about the use and treatment of experimental animals.

Transmission electron microscopy

Semi- and ultra-thin sections were analyzed at the Electron Microscopy Core Facility (Universidad Complutense, Madrid, Spain). Images were captured using a JEOL microscope (JEM-2000 FX).

Microarray labeling

Total RNA was isolated from four biological replicates of pre- and post-senescence MSC and from TMC using the TriReagent Solution (Sigma). RNA was purified with MegaClear (Ambion), and integrity confirmed on an Agilent 2100 Bioanalyzer (Agilent Technologies). Total RNA (1.5 μg/sample) was amplified using the Amino Allyl MessageAmp aRNA kit (Ambion) to obtain 15–60 μg of amino-allyl amplified RNA (aRNA); mean aRNA size was 1500 nucleotides. For each sample, aRNA (2.5 μg) was labeled with one aliquot of Cy3 or Cy5 Mono NHS Ester (CyDye Post-labeling Reactive Dye Pack, Amersham) and purified using the Amino Allyl MessageAmp aRNA kit. Cy3 and Cy5 incorporation were measured using 1 μl probe in a Nanodrop spectrophotometer (Nanodrop Technologies). For each hybridization, 80–100 pmol each of Cy3 and Cy5 probes were mixed, dried by speed-vacuum, and resuspended in 9 μl RNase-free water. Labeled aRNA was fragmented by adding 1 μl of 10× fragmentation buffer (Ambion) and incubation (70 °C, 15 min). The reaction was terminated with 1 μl stop solution (Ambion).

Slide treatment and hybridization

Slides containing 22,102 annotated genes corresponding to the human 70-mer oligonucleotide library (V2.2) (Qiagen-Operon) were obtained from the Genomics and Microarray Laboratory (Cincinnati University). Information on printing and the oligo set are found at http://microarray.uc.edu. Slides were prehybridized (42 °C, 45–60 min) in 6× saline sodium citrate buffer (SSC), 0.5% sodium dodecyl sulfate (SDS) and 1% bovine serum albumin (BSA), then rinsed 10 times with distilled water. Fragmented Cy3 and Cy5 aRNA probes were mixed (80–100 pmol each) with 10 μg PolyA (Sigma) and 5 μg human Cot-DNA (Invitrogen), and dried in a speed-vacuum. Each probe mixture was then resuspended to a final volume of 60 μl in hybridization buffer (50% formamide, 6× SSC, 0.5% SDS, 5× Denhardt's solution). Probes were denatured (95 °C, 5 min) and applied to slides, then incubated (48 °C, 16 h) in hybridization chambers (Array-It; Telechem International) in a water bath. After incubation, slides were washed twice with 0.5× SSC, 0.1% SDS (5 min each), three times with 0.5× SSC (5 min), and once in 0.05× SSC (5 min), then dried by centrifugation (563×*g*, 1 min). Images from Cy3 and Cy5 channels were equilibrated and captured with an Axon 4000B scanner, and spots quantified using GenePix 5.1 software.