

Concerted immune evasion and intrinsic inflammation as consequence of *in vitro* neoplastic transformation of mesenchymal stem cells

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ABSTRACT

Immune escape is one of the essential properties of tumors. However, there are no evidences discriminating if the establishment of the immune suppressor phenotype is exclusively determined by the immune system pressure on tumor progression, or intrinsically supported by the events leading to neoplastic transformation. To unravel this dichotomy, we used two *in vitro* models based on the sequential transformation of mesenchymal stem cells (MSC). Malignant transformation reinforces the natural immune escape properties of MSC, mediated by the reduction of membrane HLA class I levels and the increasing inhibitory capacity on T cells. It was evidenced that as a consequence of transformation, there is a transition in the natural inhibitory effect of MSC on T-cell proliferation from an inducible mechanism depending on IFN- γ signaling and mediated by indoleamine 2,3-dioxygenase towards a constitutive mechanism involving TGF- β , HGF, COX-2 and PDL-1. It was found that the increased expression of the inflammatory mediators IL-1 β and PGE $_2$ caused by the neoplastic transformation of MSC supports the immune suppressor properties of these cells. Our results indicate that oncogenic signals are able to concert intrinsic inflammation and immune escape, independently of the selective pressure of the immune system. This research granted the 2015 Award of the Cuban National Academy of Sciences.

Keywords: mesenchymal stem cells, neoplastic transformation, inflammation, immune evasion, T cell response, cancer

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RESUMEN

Concertación de la inmuno-evasión y la inflamación intrínseca como consecuencia de la transformación neoplásica *in vitro* de células madre mesenquimales. El escape a la vigilancia del sistema inmunológico es considerado una de las principales características de los tumores. Sin embargo, aún no existen evidencias que permitan discernir si el establecimiento del fenotipo inmunosupresor en las células tumorales es una consecuencia exclusiva de la influencia del sistema inmune durante la progresión tumoral o si hay una contribución intrínseca de los eventos moleculares que conducen a la transformación neoplásica. Para abordar este aspecto, se emplearon dos modelos experimentales de transformación secuencial *in vitro*, basado en células madres mesenquimales (MSC). En el presente trabajo se demuestra que la transformación maligna refuerza las propiedades naturales de inmuno-evasión de las MSC mediante la reducción de los niveles de HLA clase I en membrana y el incremento de la capacidad de inhibir la proliferación de linfocitos T. Se evidenció que como consecuencia de la transformación ocurre una transición desde un mecanismo de inhibición de la proliferación de los linfocitos T inducible por IFN- γ y mediado por indoleamina 2,3-dioxigenasa a uno constitutivo que involucra TGF- β , HGF, COX-2 y PDL-1. Este trabajo demuestra que el incremento de la expresión de los mediadores de la inflamación IL-1 β y PGE $_2$ como consecuencia de la transformación neoplásica de las MSC, sostiene la capacidad inmunosupresora de estas células. Estos resultados indican que las señales oncogénicas son capaces de concertar la inflamación intrínseca y la evasión a la inmunovigilancia, independientemente de la influencia selectiva del sistema inmune. Esta investigación mereció el Premio de la Academia de Ciencias de Cuba en el 2015.

Palabras clave: células madre mesenquimales, transformación neoplásica, inflamación, evasión inmune, respuesta de células T, cáncer

Introduction

In the tumor microenvironment, one of the external pressures acting on tumors derived from the immunological surveillance [1]. However, according to the immunoediting theory, the immune system not only destroys cancer cells, but it could also promote cancer progression through the selection of cells variants capable to progress in an immunocompetent host [2]. The knowledge accumulated over the last two decades has evidenced how complex is the influence of the immune system on tumor development. In this sense, new evidences coming from basic and clinical research defines cancer as an inflammatory disease

[3]. The prevalence of a chronic inflammatory state associated to cancer, has been defined as an enabling characteristic that fosters multiple hallmarks of cancer [4].

Chronic inflammation can influence the early phases of tumor progression as an extrinsic event, but also the genetic events leading to malignant transformation can establish an intrinsic pro-inflammatory state even in tumors without preexisting external inflammatory conditions [5]. Currently, there are no evidences that allow discriminating if the immunosuppressive phenotype of tumor cells results

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exclusively from the extrinsic pressure of the immune system during tumor progression, or if there is an intrinsic contribution of the molecular events that lead to neoplastic transformation. Additionally, whether inflammatory mediators contribute to concert such immunosuppressive feature is an unsolved and clinically relevant issue.

In order to answer these questions, we developed an *in vitro* step-wise neoplastic transformation model, based on the decreased expression of the p53 tumor suppressor gene and the expression of the oncogenic variant H-Ras^{V12} in murine bone marrow-derived mesenchymal stem cells (MSC). Moreover, we selected another experimental transformation model, created by Funes *et al.* in 2007, in which five sequential genetic modifications were added to human MSC [6]. These are valuable models because MSC naturally exert a plethora of modulatory effects on immune cell functions [7]. In this work we demonstrated how neoplastic transformation of MSCs inherently reinforces the immune escape properties on these cells by inducing molecular mediators of inflammation.

Results

Implementing a model of neoplastic transformation in MSCs and its effects on T cell immune response

A neoplastic model was established by a sequential infection with lentiviral vectors encoding the protein E6 from the human papillomavirus serotype 16, which leads to the decreased expression of p53 tumor suppressor gene, and the H-Ras^{V12} oncogenic variant. It was demonstrated that transformation potentiates the inhibitory effect of MSCs on T cell proliferation, increasing the frequency of apoptotic T cells, but not reducing the expression of the CD25 activation marker on the cell membrane. The malignant transformation led to a loss of dependency on nitric oxide for the immunosuppression of T cells, while increase the constitutive production of the inflammatory molecule PGE-2. In addition, we show for the first time that MSC can regulate the differentiation and function of myeloid derived suppressor cells (MDSC), which is enhanced in neoplastic cells [8].

Effect of the neoplastic transformation of human MSC on the immune evasive properties and the expression of molecular mediators of inflammation

We implemented the *in vitro* model of sequentially transformed human MSC developed by Funes *et al.* in 2007. Malignant transformation in this model increased the immune evasive properties of human MSC, either by reducing the membrane HLA class I levels or by increasing the anti-proliferative capacity of human MSC over T cells. Furthermore, the signaling cascade through the IFN- γ receptor was globally

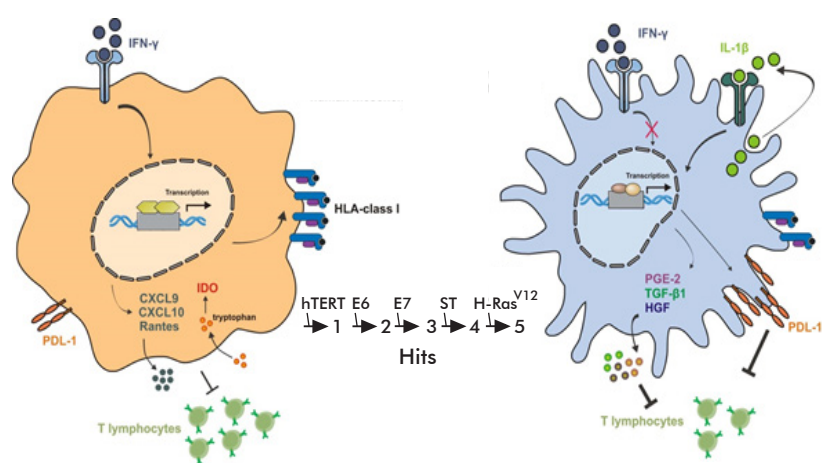


Figure 1. Neoplastic transformation of human MSC leads to an immune evasive and pro-inflammatory phenotype. Five successive genetic modifications (Hits) in normal human MSCs conducts to insensitivity to IFN- γ that influence HLA-class I expression and dampen the mechanism of T-cell suppression of normal MSC (IDO, CXCL-9, CXCL-10, Rantes). Malignant transformation leads to an autocrine inflammatory loop of IL-1 β , which increases the suppression of T-cell proliferation by supporting the constitutive expression of the immunosuppressive molecules PDL-1, TGF- β 1, HGF and PGE-2. IDO: Indoleamine 2,3-dioxygenase; PDL-1: Programmed death ligand-1; HGF: Hepatocyte growth factor; TGF- β 1: Transforming growth factor- β 1; PGE-2: Prostaglandin E2.

affected in neoplastic human MSC. In fact, transformation led to a transition from a suppressor mechanism over T cell proliferation, inducible by IFN- γ and mediated by indoleamine 2,3-dioxygenase, towards a constitutive mechanism that relied upon IL-1 β and involve either secreted or membrane bound molecules. The increased expression of IL-1 β due to transformation sustains the immunosuppressive properties of neoplastic human MSC. Our results demonstrate that oncogenic activation orchestrates inflammation and immune escape, independently of any need for immunoediting in the tumor microenvironment [9].

Relevance of the study

In this work it was evidenced for the first time that genetic alterations leading to neoplastic transformation are capable of enhancing tumor evasion from immune surveillance, even in the absence of the selective influence exerted by the immune system in the tumor microenvironment. Moreover, it was demonstrated that transformation of MSC increases their immunosuppressive ability through a transition from an inducible mechanism extrinsically regulated towards a constitutive mechanism that depend on intrinsic inflammatory molecules. This is the main contribution of our work to the knowledge of tumor biology.

On a broader perspective, our study supports the relevance of controlling the cancer associated inflammation as a strategy to mitigate tumor immune escape. Particularly, it was evidenced that the IL-1 β inflammatory cytokine could be a possible target for future antitumor therapies.

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