Oncogenic Transformation Can Orchestrate Immune Evasion and Inflammation in Human Mesenchymal Stem Cells Independently of Extrinsic Immune-Selective Pressure

Alex Miranda1, Juan M. Funes2, Nilda Sánchez1, Celia M. Limia1,3, Mónica Mesa1, Sergio A. Quezada2, Rolando Pérez1, and Joel de León1

Abstract

Immune escape is a hallmark of cancer, but whether it relies upon extrinsic immune-selective pressure or is inherently orchestrated by oncogenic pathways is unresolved. To address this question, we took advantage of an in vitro model of sequentially transformed human mesenchymal stem cells (hMSC). Neoplastic transformation in this model increased the natural immune evasive properties of hMSC, both by reducing their immunogenicity and by increasing their capacity to inhibit mitogen-driven T-cell proliferation. We also found that IFNγ signaling was globally affected in transformed hMSC. As a consequence, the natural inhibitory effect of hMSC on T-cell proliferation switched from an inducible mechanism depending on IFNγ signaling and mediated by indoleamine 2,3-dioxygenase to a constitutive mechanism that relied upon IL1β involving both secreted and membrane-expressed molecules. After transformation, increased IL1β expression both sustained the immunosuppressive properties of hMSC and increased their tumorigenicity. Thus, in this model system, IL1β acted as intrinsic inflammatory mediator that exerted an autocrine influence on tumor growth by coordinately linking immune escape and tumorigenicity. Collectively, our findings show how oncogenes directly orchestrate inflammation and immune escape to drive the multistep process of cancer progression, independently of any need for immunoeediting in the tumor microenvironment. Cancer Res; 75(15); 3032–42. ©2015 AACR.

Introduction

Genetic and epigenetic somatic lesions that sustain the hallmarks of cancer (1) are selected by intrinsic and extrinsic pressures, including immune surveillance, which persistently operate on malignant cells during cancer progression. The theory of cancer immunoediting postulates that effector mechanisms of the immune system exert a dual role, eliminating cancer cells and promoting cancer progression through the selection of those cells whose genetic alterations allow tumor to progress in immunocompetent host (2).

The molecular links between cancer cells and immune system components extend beyond immune surveillance evasion. Chronic inflammation can trigger the earliest stages of cell transformation (3), whereas intrinsic inflammatory programs prompted by genetic events influence genetic instability and enable the rest of cancer hallmarks, leading to neoplastic progression (1, 4). Molecular inflammatory mediators can contribute to tumor immune escape, mainly by recruiting regulatory immune cells that suppress protective tumor immunity (5).

However, whether immune-suppressive capacities of cancer cells evolve along the multistep development of human tumors independently of immune-extrinsic pressure and cancer-intrinsic inflammation contributes to concert such immunosuppressive feature are unsolved and clinically relevant issues. To address these questions, we took advantage of an in vitro experimental model in which five-stepwise genetic modifications convert normal human mesenchymal stem cells (hMSC) into neoplastic cells (6). This is a valuable model because hMSC naturally exert a plethora of modulatory effects on immune cell functions (7, 8). These cells also contribute to the progression of certain tumors either modifying the microenvironment (9, 10) or behaving as tumor-initiating cells (11, 12). In fact, using a more simplistic model of transformation based on murine MSC, we have preliminary evidence that in vitro–induced neoplastic transformation modifies the relationship between MSC and lymphocytes (13). In the human model, the induced sequential transformation generates incomplete or 5 hits fully transformed MSC lines by sequential and accumulative infections with retroviruses encoding for hTERT (1 hit), HPV-16 E6 (p53 kd; 2 hits) and E7 (pRb kd; 3 hits) proteins, SV40 small T antigen (c-myc activation; 4 hits), and H-RasV12 (H-RAS activation; 5 hits).

Materials and Methods

Cell culture

Peripheral blood mononuclear cells (PBMC) isolated by Ficoll-Paque Plus density gradient (cat. no. 17-1440-02; GE Healthcare...
Life Science) from buffy coats of healthy blood donors were cultured in RPMI-1640 medium (Gibco) supplemented with 10% FCS, 2 mmol/L glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 50 μmol/L β-mercaptoethanol. hMSC lines (7) were grown in DMEM medium (Gibco) supplemented with 10% FCS. In some experiments, 2 × 10^5 hMSC were seeded in 6-well plates and cultured for 72 hours with medium containing 1.5 mg/mL IFNγ (cat. no. 554617; BD Pharmingen) and/or 1.5 mg/mL TNF-α (cat. no. 210-TA-050; R&D Systems), or 1:2 diluted supernatant collected from PBMC activated with 5 μg/mL of Concanavalin A (ConA; cat. no. 234567; Merck Millipore).

**Proliferation assays**

PBMC (10^6) labeled with 0.5 μmol/L of carboxyfluorescein diacetate succinimidyl ester (CFSE; cat. no. C34554; Life Technologies) were cocultured in 24-well plates with supernatant derived from hMSC or with different amounts of hMSC pretreated or not with 25 μg/mL of mitomycin C (cat. no. M4267; Sigma). When required, Transwell chambers (0.4-μm pore size; BD Falcon) were used. Lymphocytes were activated with ConA (5 μg/mL), harvested after 72 hours, and stained with phycoerythrin (PE)-conjugated anti-CD3 Ab (1:100 dilution, cat. no. 12003842; eBioscience) before acquisition in a Gallios flow cytometer (Beckman Coulter).

**FACS**

Cell surface expression of HLA-ABC, PD-L1, and IL1R1 was measured by direct immunofluorescence using FITC-conjugated anti–HLA-ABC antibody (1:200 dilution; cat. no. 555552; BD Pharmingen), APC-conjugated anti-CD274 (B7-H1, PD-1) antibody (1:100 dilution; cat. no. 329707; Biolegend), and APC-conjugated anti-hIL1R1 (1:100 dilution; cat. no. FAB269A; R&D System), respectively. For intracellular staining, cells were fixed and permeabilized with a Golgi Stop Kit according to the manufacturer’s protocol (BD Pharmingen), and then stained with PE-conjugated anti-Rantes Ab (1:100 dilution; cat. no. 554732; BD Pharmingen), PE-conjugated anti-II-β Ab (1:100 dilution; cat. no. 554732; eBioscience), or isotype controls. Cell acquisition was analyzed with FlowJo 7.2.2 software. In each case, AMFI values were calculated as [MFI (relevant Ab) – MFI (isotype control Ab)].

**Western blotting**

Cell lysates (30 μg protein) were fractionated on 10% to 15% SDS-polyacrylamide gradient gels (Bio-Rad) and transferred to Hybond-P membranes (Amersham Pharmacia). Membranes were blocked with 5% milk in TBS containing Tween 0.1% and incubated with: mouse anti-IDO Ab (1:500 dilution; cat. no. 05-840; Millipore); rabbit anti–HLA-ABC Ab (1:500 dilution; cat. no. SC-52810; Santa Cruz Technologies); rabbit anti-STAT1 (1:1,000 dilution; cat. no. 9127; Cell Signaling); rabbit anti–p-STAT1 (Tyr701; 1:1,000 dilution; cat. no. 7649; Cell Signaling); mouse anti-II-β (1:300 dilution; cat. no. 552289; BD Biosciences); rabbit anti–β-Actin Ab (1:1,000 dilution; cat. no. 49671; Cell Signaling). Horse anti-mouse IgG (1:1,000 dilution; cat. no. 7076; Cell Signaling) or goat anti-rabbit IgG (1:1,000 dilution; cat. no. 7074; Cell Signaling) conjugated to horseradish peroxidase was used for detection, and visualized using enhanced chemiluminescence detection system (Santa Cruz Technologies).

**ELISA**

Concentration of IL2, TGFβ1, PGE-2, and IL1β in culture supernatants was measured by ELISA assays following the manufacturer’s instructions: IL2 (cat. no. 88-7025; eBioscience), TGFβ1 (cat. no. DY1679; R&D Systems), PGE-2 (cat. no. KGE004B; R&D Systems), and IL1β (cat. no. 557953; BD Biosciences).

**Tumorigenicity assays**

For anchorage-independent growth of hMSC, 10^4 cells were transferred to 2 mL of culture medium containing 0.35% low melting point agarose (Sigma). Cells were seeded in triplicate in 6-well plates containing a layer of solidified 0.6% agarose. Fresh medium was added every 3 days. Colonies were photographed at ×40 magnification on day 12. For in vivo tumor formation assay, 5 × 10^5 5 hits hMSC were injected subcutaneously in both flanks of 8-week-old athymic nude mice (CENPALAB). Tumors were explanted and incubated for 30 minutes at 37°C with 1 mg/mL of collagenase (cat. no. 9001-12-1; Worthington Biochemical Corporation), 0.5 mg/mL of DNase I (cat. no. AMPD1; Sigma), and 0.1 mol/L of EDTA pH 7.2 (Sigma). Tumors were homogenized to a single-cell suspension and placed into culture conditions. All animal handling and in vivo experiments were performed in accordance with CIM’s guidelines.

**Statistical analyses**

Statistical significance (P < 0.05) was determined by an unpaired two-tailed Student t test for single comparisons and one-way ANOVA with the Dunnett post-hoc test for multiple comparisons. Statistical analyses and graphs were developed using SPSS software and GraphPad Prism 5.0, respectively.

**Results**

Oncogenic transformation strengthens natural immune-evasive properties of hMSC

To assess the inherent contribution of oncogenic pathways on the natural immune-evasive capabilities of hMSC, we focused our attention on comparing hMSC from 2 to 5 hits and explored the effect of transformation on cell immunogenicity and active immunosuppression. We followed the transcription and protein expression of HLA class I during the transformation process. Although HLA-A and B alleles are particularly sensitive to inactivation of PRB function (Fig. 1A), the mRNAs of each HLA alleles and the total HLA-ABC protein levels in 2 and 5 hits cells were barely modulated (Fig. 1A), the mRNAs of each HLA alleles and the total HLA-ABC protein levels in 2 and 5 hits cells were barely modulated. However, HLA class I expression on the plasmatic membrane of fully transformed cells was 50% reduced (Fig. 1B). During transformation, a Gene Set Enrichment Analysis (GSEA) reveals that overall expression profile of genes involved in HLA class I antigen-presenting machinery (APM; Supplementary Table S1) was significantly downregulated (P < 0.001). Hierarchical clustering analysis identified a set of genes downregulated at the late stages of transformation (Fig. 1C). Transcriptional repression of β2-microglobulin, PDIA3 (ERp57), ERP2, and NPEPPS can impair membrane expression of HLA-ABC on neoplastic hMSC (Supplementary Fig. S1).

Normal hMSC affect proliferation and effector functions of T lymphocytes (14). We examined the inhibitory effect of hMSC over mitogen-triggered T-cell proliferation following stepwise...
Figure 1. \textit{In vitro} stepwise transformation strengthens natural immune-evasive properties of hMSC.

A, left, HLA class I mRNA alleles and total HLA-ABC protein expression determined by qRT-PCR (\(*\), \(P < 0.01\); \(***\), \(P < 0.001\); \(n = 3, \text{mean} \pm \text{SEM}\)) and Western blot assay (right; values are band intensities relative to 2 hits) n.s., nonsignificant. B, cell surface expression of total HLA class I molecules evaluated by FACS analysis expressed as percentages of positive cells (\(\pm \text{SEM}\)), and \(\Delta\text{MFI}\) values isotype controls staining are shown as filled histograms. C, hierarchical clustering analysis of \(Z\) score data and GSEA \((P < 0.001)\) of genes related to HLA class I APM. The heatmap summarizes the average of three samples from each cell line. Underlined gene symbols represent differentially expressed genes for 5 hits versus 2 hits comparison. D, effect of sequentially transformed hMSC lines on T-cell proliferation. CFSE-stained PBMC were cocultured with hMSC lines (2 to 5 hits) at 1:25 (MSC:PBMC) ratio. The percentage of CD3\(^+\) proliferating cells was evaluated by FACS after 72 hours of stimulation with ConA.
transformation. A progressive increase on the suppressive effect of hMSC was observed at 1:25 hMSC:PBMC ratio. Maximum effect was seen with 5 hits cells with up to 50% inhibition of CD3$^+$ lymphocytes proliferation comparing to stimulated PBMC-positive controls (Fig. 1D). This effect was confirmed at different hMSC:PBMC ratios using mitomycin C–pretreated hMSC cells (Supplementary Fig. S2). In addition, although normal hMSC do not affect T-cell activation (15), a reduction of IL2 secretion and CD25 expression occurs when PBMC were cocultured with hMSC following transformation events (Supplementary Fig. S3). Collectively, these data suggest that transformation can develop immune-evasive properties on hMSC, not as a consequence of extrinsic immune pressure but as an intrinsic result of oncogenic pathways.

Insensitivity to IFN$\gamma$ signaling influences the immunogenicity and the mechanism of T-cell suppression on transformed hMSC

Normal hMSC affect T-cell function through the concerted action of IDO and T-cell–specific chemokines, which are induced by inflammatory cytokines on hMSCs (16, 17). We addressed whether neoplastic transformation has the intrinsic capability to either potentiate the natural mechanism of immune dysfunction produced by hMSC or induce an alternative mechanism.

When the supernatant collected from ConA-activated PBMC was added to hMSC lines, a significant decrease in inducible expression of IDO (Supplementary Fig. S4A), CXCL9, CXCL10, and Rantes (Supplementary Fig. S4B) was detected on transformed cells. As described for normal hMSC (16), exogenous IFN$\gamma$ elicited IDO expression in 2 hits cells (Fig. 2A), while
concomitant signaling of both IFNγ and/or TNFα inflammatory cytokines was required for chemokine induction (Fig. 2B). Conversely, the inducible expression of IDO was reduced in 4 hits cells, as shown by Western blotting, whereas it was abrogated in the fully transformed 5 hits hMSC. CXCL9 and CXCL10 mRNA levels were also impaired in neoplastic hMSC (Fig. 2B). Therefore, immunosuppression of T cells mediated by IDO and induced in hMSC by inflammatory cytokines is not reinforced but abrogated in transformed hMSC.

The apparent lack of sensitivity of neoplastic hMSC to IFNγ could reflect a balance between the disadvantage of reducing the production of IDO, as a mediator of immunosuppression, or the advantage of controlling the immunogenicity by limiting the ability of IFNγ signaling to upregulate the transcription of the APM. In fact, transcription of individual HLA class I alleles induced by IFNγ exposure is higher in partially than in fully transformed cells (Supplementary Fig. S5). The total protein level (Fig. 2C) and membrane expression (Fig. 2D) of HLA-ABC was unmodified on 5 hits hMSC treated with this cytokine.

Because transformed hMSC failed to elicit a range of IFNγ-inducible genes, suggesting unresponsiveness to IFNγ, we tested the hypothesis that the IFNγ signaling pathway is defective in

Figure 3. IFNγ signaling pathway is affected on progressively transformed hMSC. A, comparative intracellular phosphorylation and nuclear accumulation of STAT1 on 2 and 5 hits hMSC lines cultured for 2 hours in control or IFNγ added medium. p-STAT1-FITC and cell nuclei counterstained with Hoechst were detected by immunofluorescence. B, Pearson colocalization coefficient for p-STAT1 and Hoechst was calculated from three fields in two independent experiments. C, Western blot analysis of STAT1 and p-STAT1 (Y701) on cells cultured for 1 hour in control or IFNγ added medium. D, STAT1 mRNA assayed by qRT-PCR on hMSC lines cultured for 72 hours in control or IFNγ added medium. Fold changes are relative to the expression of the gene in each hMSC line versus 2 hits cells cultured in control medium (n = 3, mean ± SEM). E, hierarchical clustering analysis of Z score data and GSEA of IFNγ signaling pathway (left; GSEA \( P < 0.001 \)) and regulators of IFNγ signaling (right; GSEA \( P > 0.05 \)). The heatmap summarizes the average of three samples from each cell line. Underlined gene symbols represent differentially expressed genes for the 5 hits versus 2 hits comparison.
Figure 4.
The constitutive ability of neoplastic hMSC to inhibit T-cell proliferation partially depends on secreted factors. A, lymphocyte proliferation induced with ConA and assayed as CFSE dilution by FACS. CFSE-stained PBMC were cocultured with hMSC lines at 1:10 (MSC:PBMC) ratio in a Transwell system for 72 hours. Data are expressed as percentage of T-cell proliferation recovery (±SEM) relative to PBMC activated in the absence of hMSC. B, percentage of proliferating T cells based on CFSE dilution by FACS. PBMC cultured in the supernatants of hMSC lines (1:2 diluted in fresh medium) were stimulated with ConA for 72 hours (n=3, mean ±SEM). Dashed line, percentage of proliferating cells in the absence of hMSC supernatants. C, comparative amount of TGFβ1, HGF, COX-2, and iNOS mRNA on 2 and 5 hits hMSC lines analyzed by qRT-PCR. Fold changes are relative to the expression of each gene versus 2 hits cells. D and E, concentration of TGFβ1 (D) and PGE-2 (E) determined in the supernatant of hMSC lines by ELISA (*, P < 0.05; **, P < 0.01; ****, P < 0.001; n=3, mean ±SEM). n.s., nonsignificant.
neoplastic hMSC. p-STAT1 homodimers rapidly accumulate in the nucleus to mediate IFNγ biologic responses (18). We assessed the nuclear accumulation of p-STAT1 at single-cell level by fluorescence microscopy. Unlike neoplastic 5 hits cells, rapid nuclear accumulation of p-STAT1 was detected in non-transformed 2 hits cells treated with IFNγ (Fig. 3A and B). Coherently, IFNγ-induced phosphorylation of STAT1 was reduced in 5 hits cells (Fig. 3C). STAT1 is an IFNγ-inducible gene (19), so we examined whether transformation abrogates inducible transcription of STAT1. As shown in Fig. 3D, expression of STAT1 mRNA is progressively reduced following transformation, reaching complete abrogation on fully transformed hMSC cells. To characterize the IFNγ receptor signaling further, we then compared the gene expression profiles of selected genes involved in this pathway (Supplementary Table S1). The GSEA indicates that transformation significantly reduced (P < 0.001) the overall expression of genes implicated in IFNγ signaling (Fig. 3E, left), and four genes other than STAT1 were significantly reduced comparing 2 versus 5 hits cells. No significant change was detected in the overall expression of negative regulatory genes (P > 0.05; Fig. 3E, right). Altogether, these data highlight a deficit in IFNγ signaling on transformed hMSC, which molds both immunogenicity and immunosuppression mechanisms in neoplastic cells.
Constitutive capacity of transformed hMSC to suppress T-cell proliferation depends on IL1β production

To address the mechanism underlying the increased inhibitory capacity of transformed hMSC, we initially evaluated whether the inhibition of T-cell proliferation depends on cellular contact. Mitogen-driven proliferation assay was performed in Transwell chambers using 1:10 hMSC:PBMC cells ratio to reach the maximal inhibitory capacity of each hMSC line (Fig. 4A, top). Partially transformed hMSC lost more than 80% of their suppressive capacity, whereas neoplastic 5 hits cells retained about 50% inhibition of lymphocyte proliferation when the Transwell system is used (Fig. 4A). This indicates that transformed hMSC increases the dependency of secreted factors to suppress T-cell proliferation. To confirm this result, PBMC were activated in culture medium containing 50% of supernatant collected from hMSC lines. Although supernatants from 4 hits cells significantly inhibited T-cell proliferation comparing with 2 hits, fully transformed cells exerted the highest suppressive effect (Fig. 4B). Hence, secreted factors partially determine the constitutive suppressive effect promoted by hMSC transformation.

To identify molecules involved in suppressing T-cell proliferation, we measured a set of molecules that are produced by normal hMSC playing a secondary role in the inhibition of T-cell proliferation (14, 20). Unlike inducible nitric oxide synthase (iNOS; Fig. 4C) and IDO (Fig. 2A) enzymes, a significant upregulation in constitutive transcription of TGFβ1, COX-2, and HGF genes was detected comparing 2 and 5 hits cells (Fig. 4C). Quantification of TGFβ1 (Fig. 4D) and PGE2 (Fig. 4E), secreted by hMSC cell lines, revealed a significant increase of both molecules due to transformation. Because these molecules are downstream targets of the IL1β signaling pathway (21), we considered the possibility that IL1β could be...
orchestrating the mechanism of T-cell suppression exerted by transformed hMSC. IL1β mRNA (Fig. 5A) and intracellular protein levels (Supplementary Fig. S6A and S6B) increase on hMSC following sequential transformation. Noteworthy, IL1β was only detected in the supernatant of fully transformed hMSC (Fig. 5B), not associated with cell lysis (Supplementary Fig. S7A) or apoptosis (Supplementary Fig. S7B). GSEA showed no significant enrichment of genes involved in IL1β signaling (Supplementary Table S1) owing to neoplastic transformation, though particular genes were upregulated comparing 5 versus 2 hits cell lines (Supplementary Fig. S8A). Interestingly, we found diminished expression of IL1 receptor 1 (IL1R1) on 5 hits cells (Supplementary Fig. S8B).

Because IL1β is only active once secreted (22), we focused on the role of IL1β in the inhibition of T-cell proliferation by 5 hits hMSC. IL1β expression was knockeddown (KD) on 5 hits hMSC by infection with lentivirus encoding IL1β shRNA (Supplementary Fig. S9A). Change in cells proliferation rate was not observed (Supplementary Fig. S9B). This manipulation restores T-cell proliferation, demonstrating a determinant role of IL1β for neoplastic hMSC-immunosuppressive property (Fig. 5C).

Neither the supernatant collected from mitogen-activated PBMC nor recombinant IFNγ recovered the inducible levels of IDO (Supplementary Figs. S10A and S11A), CXCL9 and CXCL10 (Supplementary Fig. S10B), STAT1 (Supplementary Fig. S11B), and HLA-ABC expression (Supplementary Fig. S11C) in IL1β KD hMSC. Therefore, IL1β seems to be unrelated to the insensitivity to IFNγ signaling caused by neoplastic transformation, whereas it is involved in the novel mechanism of immune suppression orchestrated during the acquisition of the neoplastic phenotype. In fact, IL1β KD hMSC have significantly reduced transcription of TGFβ1, HGF, and COX-2 mRNA (Fig. 5D) as well as secreted levels of TGFβ1 (Fig. 5E) and PGE2 (Fig. 5F) when compared with the scramble shRNA control. To ponder the contribution of such soluble factors to IL1β-concerted immunosuppression, PBMC were mitogen activated in culture medium containing 50% of culture supernatant collected from IL1β KD or scramble shRNA cell lines. Supernatant from IL1β KD cells exhibited only a partially reduced capacity to inhibit T-cell proliferation (Fig. 5G), suggesting that beside secreted molecules, a complementary mechanism controlled by IL1β is mediating the suppression of T lymphocytes proliferation.

Signaling through the programmed death-1 receptor (PD-1) induced by its ligands PD-L1 and PD-L2 modulates the T-cell response (23). Recent evidence demonstrates that IL1β can induce the upregulation of PD-L1 (24). Fully transformed hMSC have significantly higher expression of PD-L1 (Fig. 5H), which is reduced as a consequence of IL1β KD (Fig. 5I), indicating that IL1β orchestrates on transformed hMSC a mechanism of immunosuppression mediated by secreted and membrane-expressed molecules.

IL1β secretion sustains the neoplastic phenotype acquired by multistep transformation of hMSC.

We further explored the role of IL1β signaling in sustaining hMSC tumorigenicity. Knockdown of IL1β in 5 hits cells dramatically reduces the number and size of colonies growing in soft agarose (Fig. 6A and B). Correspondingly, only one out of six tumors grew in athymic nude mice inoculated with IL1β KD 5 hits hMSC (Fig. 6A). The sensitivity of tumorigenic phenotype to IL1β was demonstrated when 5 hits lines expressing different levels IL1β were obtained and tested in a colony formation assay. We found a strong correlation between the production of IL1β and the number of growing colonies, revealing the sensitivity of neoplastic hMSC to an IL1β-mediated autocrine loop (Fig. 6C). To address whether IL1β production is sustained in vivo, we compared IL1β expression on 5 hits hMSC and explanted tumors. Gene expression microarray analysis (Fig. 6D, top) and intracellular staining (Fig. 6D, bottom) demonstrate that in vivo growing cells preserve IL1β expression. Transcription of IL1β gene was highest on cells isolated from tumors, whereas explanted cells cultured in vitro have as much intracellular expression of IL1β as 5 hits hMSC. It suggests that IL1β expression was prompted into the tumor microenvironment.

Discussion

Immunity exerts dual protective and tumor-progressive actions on developing tumors. Escape of cancer cells from immune control has been assumed as a direct consequence of immune pressure (25), involving reduced presentation of immunogenic antigens (26) and a plethora of active immune-suppressive strategies (27). Based on this hypothesis, immune effector mechanisms select for expansion of those cancer cells possessing an effective counterattack mechanism. However, taking advantage of a neoplastic transformation model created by in vitro-induced sequential and accumulative genetic alterations on hMSC (6), we showed that immune-evasive mechanisms can evolve along the multistep process of neoplastic transformation independently of immune-selective pressure. Progressive transformation reduces hMSC immunogenicity, evidenced as transcriptional downregulation of the APM and reduced expression of HLA class I molecules at the plasmatic membrane, one of the best characterized mechanism used by tumors to impair antigen presentation and avoid T-cell recognition (28).

Stepwise transformation progressively increases the natural inhibitory effect of hMSC on T-cell proliferation. The inhibitory effect switches from a mechanism inducible and regulated by extrinsic inflammatory signals to another that is constitutive and regulated by an autocrine inflammatory loop. Neoplastic hMSC lose the sensitivity to the canonical IFNγ signaling pathway evidenced by altered STAT1 phosphorylation and migration to the nucleus, associated with transcriptional impairment of IFNγ signaling–related genes. This benefits cancer progression, considering the relevance of IFNγ in cancer immunosurveillance (29). In fact, potential overexpression of APM and secretion of chemokines recruiting T cells (i.e., CXCL9, CXCL10, and RANTES) influenced by IFNγ is dampened. In parallel, halt of IFNγ signaling abrogates the inducible expression of IDO, a mediator of the natural suppressive capacity of hMSC (16). Hence, neoplastic hMSC sustain the suppressive effect by gaining a constitutive expression of TGFβ, HGF, PGE2, and PD-L1, dissociating the suppressive effect from the extrinsic inflammatory influence. Along with upregulation of HLA-A and HLA-B alleles, inhibition of p18b expression (3 hits cells) reduces the expression of PD-L1. It suggests that achievement of oncosenescence depends on circumventing incomplete transformation stages that are highly sensitive to immune attack during the multistep process of transformation. It is well established that oncoencephalization leads to a protumoral intrinsic inflammatory program (30). In our model, we detected increased transcription and intracellular expression of...
IL1β following sequential transformation. Secretion of this cytokine was only detected on fully transformed 5 hits cells, suggesting that early transformation steps cooperate on transcription and translation of IL1β, whereas H-RAS-induced signaling activates the secretion of the mature cytokine. The link between RAS pathway and the consequent secretion of IL1β, which, in turn, upregulates HIF1α via COX2 has been described (31, 32). Correspondingly, an IL1β-sensitive increase in PGE2 secretion occurs on 5 hits cells, whereas IL1β expression is upregulated on hMSC growing as tumors in in vivo hypoxic conditions. Furthermore, secreted IL1β induces an autocrine inflammatory loop on neoplastic hMSC, which directs the suppression of T-cell proliferation by supporting the constitutive expression of immunosuppressive molecules. In fact, either secreted or membrane-expressed suppressor factors prompted by neoplastic transformation on hMSC are sensitive to IL1β signaling (21, 24). Of note, although H-RAS constiutive activation favors IL1β secretion and p-STAT-1 (Ty701) inhibition, IL1β is not responsible for IFNγ-halted sensitivity induced by transformation on hMSC. It suggests that transformation induces a signaling bias: reducing the extrinsic inflammation-induced immunogenicity and increasing the intrinsic inflammation-induced immune suppression.

Previous experimental models highlighted the relevance of host-derived IL1β on chemical carcinogenesis (33). Also, its capacity to mobilize suppressive cells into the tumor microenvironment has been seen in genetically manipulated animals (34). The relevance of IL1β for cancer progression in humans has been demonstrated in melanoma (24) and non–small cell lung cancer (NSCLC) patients (35). The former study demonstrated that IL1β secreted by tumors carrying the BRAF mutation V600E promotes a pro-angiogenic inflammatory loop or promotes a different suppressive loop on neoplastic hMSC, which directs the suppression of T-cell proliferation and p-STAT-1 (Ty701) inhibition, IL1β is not responsible for IFNγ-halted sensitivity induced by transformation on hMSC. It suggests that transformation induces a signaling bias: reducing the extrinsic inflammation-induced immunogenicity and increasing the intrinsic inflammation-induced immune suppression.

Intrinsic Inflammation and Immunomodulation, integrating immunity’s roles in cancer suppression and promotion. Science 2011;331:1565–70.


Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: A. Miranda, R. Pérez, J. de León
Development of methodology: A. Miranda, N. Sánchez, C.M. Limia
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Miranda, J.M. Funes, N. Sánchez, C.M. Limia, M. Mesa
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Miranda, M. Mesa, R. Pérez, J. de León
Writing, review, and/or revision of the manuscript: A. Miranda, J.M. Funes, N. Sánchez, C.M. Limia, S.A. Quezada, R. Pérez, J. de León
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.M. Funes
Study supervision: J. de León

Acknowledgments
The authors thank Belinda Sánchez and Salvador Moncada for their useful comments about this work. CIM’s animal house technicians for excellent assistance, Janet Fernihough for editing, and Yasser Almeida, Julio Fernandez, and Xitally Popa for technical help.

Grant Support
This work was financially supported by CIM, complemented with BIF and EMBO travel grants.

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Received November 7, 2014; revised April 20, 2015; accepted May 12, 2015; published OnlineFirst June 11, 2015.
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doi:10.1158/0008-5472.CAN-14-3276

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