Cancer/Testis Antigen Expression on Mesenchymal Stem Cells Isolated from Different Tissues

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Abstract. *Background/Aims: The expression of cancer/testis antigens (CTAs) on additional normal tissues or stem cells may restrict their use as cancer targets. The objective of the present study was to evaluate the mRNA levels of some CTAs in a variety of tissues. Materials and Methods: mRNA of pericytes, fibroblasts and mesenchymal stem cells (MSCs) derived from adult and fetal tissues, human umbilical vein endothelial cells, MSC-derived adipocytes, selected normal tissues and control cancer cell lines (CLs) were extracted and quantitative polymerase chain reaction was performed for MAGED1, PRAME, CTAG1B, MAGEA3 and MAGEA4. Results: MAGED1 was expressed in all normal tissues and cells evaluated. CTAG1B was expressed at levels comparable to control CLs on MSCs derived from arterial, fetal skin, adipose tissue and saphenous vein, heart, brain and skin tissues. MAGEA4 was detected only in fibroblasts and differentiated adipocytes from MSCs, at levels comparable to the control CLs. Conclusion: The potential use of CTAs in immunotherapy should take into account the potential off-target effects on MSCs.*

Over the last decades, researchers have searched for tumor antigens with no or highly restricted expression in normal tissues in order to explore their potential as immunotherapeutic targets for cancer vaccines or antibody-based therapies. After large-scale screening in a variety of cells, some antigens were found to be expressed in a range of malignant cells and in testis. These antigens are known as cancer/testis antigens (CTAs) and have emerged as potential targets for antigen-specific cancer therapies (1). In addition to being expressed in a wide variety of tumors, their expression may also be observed in a restricted set of normal tissues, including germ cells of the testis, fetal ovary and placental tissues. Some immature cells, such as spermatogonia and oogonia cells, placental cells such as trophoblasts and non-gametogenic tissues, such as pancreas, liver and spleen may also display CTA expression, although at levels far below those of germ cells (2). Previously, it was reported that CTAs are expressed in both fetal and adult mesenchymal stem cells (MSCs) of bone marrow, but with down-regulated expression in differentiated cells as adipocytes and osteocytes (3). Originally isolated from bone marrow, MSCs are defined as adherent and fibroblastoid-like cells with a capacity of *in vitro* differentiation into adipocytes, osteosblasts and chondrocytes (4). However, MSCs have also been isolated from a variety of adult tissues such as the placenta, umbilical cord blood, umbilical cord tissue, adipose tissue and dental pulp and fetal tissues such as the spleen, pancreas, kidney and lung (5-8). In addition, the identification of MSCs throughout virtually all tissues of the body has been ascribed to their localization in the walls of the vasculature (9, 10). Although MSCs from different tissues resemble each other, some genes may be differentially expressed according to their origin (9, 11). For this reason, the aim of this study was to evaluate the CTA expression in MSCs derived from different sites. The identification of CTAs in specific MSCs is important for cell therapy and cancer treatment because CTA-based cancer vaccines may generate off-target effects on MSCs expressing CTAs.

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Materials and Methods

Isolation, culture and differentiation capacity of cells. The isolation, culture and characterization of the cells used in the present study are detailed in a previous publication (9). In brief, fetal tissues were obtained during autopsy of aborted fetuses. Normal skin and adipose tissue were obtained from diagnostic biopsies. Segments of the saphena vein were obtained from patients submitted to heart surgery, as described previously (12). Bone marrow aspirates and

umbilical cord vein were collected from donors and MSCs were obtained as described previously (11, 13, 14). Pericytes were obtained from human retina (15) and fibroblasts were obtained from adult abdominal skin, fetal muscle fascia, and foreskin tissues (16). Human umbilical vein endothelial cells(HUVECs) were isolated, as originally described (17). After the isolation of MSCs, pericytes and fibroblasts, immunophenotypical characterization and differentiation potential were evaluated as described previously (12). The cells from third and fifth passages were used in all experiments, except for foreskin, where the cells from the eleventh and seventeenth passages were used.

RNA extraction and cDNA synthesis. RNA from MSCs obtained from different tissues (pericytes, n=2; fibroblasts, n=5; MSCs derived from adult and fetal tissues, n=5, 8, respectively; HUVECs, n=1; MSCs-derived adipocytes, n=1; and six selected normal tissues) were extracted using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA) and analyzed for integrity by 1% agarose gel electrophoresis. RNA extracted from cell lines (CLs), expressing CTAs established from metastatic (MZ2-MEL and LB373) and primary (WM1552 and WM793) melanomas and from erythroleukemia (K562) were used as controls. One microgram of DNAse-treated RNA (DNAse I Amplification Grade; Invitrogen) was reverse-transcribed into cDNA with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to manufacturer's recommendations.

Real-time quantitative polymerase chain reaction (PCR). PCR amplification was carried out in 96-well plates with optical adhesives on an ABI Prism 7300 sequence detection system (Applied Biosystems) using a Taqman PCR assay for MAGEA1 (HS00607097), MAGEA3 (HS00366532), MAGEA4 (HS00365979), MAGED1 (HS00199603), CTAG1B (HS00265824) and PRAME (HS00196132) genes. For each run, cDNA samples and a no-template control were all assayed in duplicate using the system's default cycle. Gene expression was normalized relative to the endogenous GAPDH (HS99999905) and the relative expression between different cell types was obtained by Pfaffl´s method (18).

Results

The differentiation capacity of MSCs, pericytes and fibroblasts and their immunophenotypical characterization were described previously by Covas *et al.* (9). Gene expression analyses by real-time PCR of the CTAs showed that among the controls, MZ2-MEL expressed the highest levels of MAGEA1, MAGEA3, MAGE-D1 and PRAME, while LB373 highly expressed MAGEA4 and CTAG1B. All CTAs had a larger expression in metastatic melanoma CLs than in primary melanoma CLs, while K562 had an intermediate expression level (data not shown).

Among the samples, MAGEA1 and MAGEA3 were detected at low levels only in MSCs derived from the saphenous vein (data not shown). MAGED1 was found to be expressed ubiquitously in all normal tissues and cells that were evaluated, setting it unambiguously apart from the other CTAs evaluated and indicating a broader function on distinct cell types of the body (Figure 1A). In addition, PRAME was expressed at low levels in MSCs from fetal testis, gonad and carotid, MSCs from umbilical cord vein, pericytes, adult and fetal fibroblasts, adipocytes and heart tissue when compared with the control CLs (Figure 1B). CTAG1B was expressed in MSCs derived from the artery, fetal skin, adipose tissue and saphenous vein and in heart, brain and skin tissues at levels comparable to those of the control CLs (Figure 1C). MAGEA4 was detected only in fetal fibroblasts and adipocytes, at levels comparable to the control CLs (Figure 1D).

Discussion

CTAs are epigenetic-regulated immunogenic molecules expressed in a wide variety of malignant tumors and restricted in immunologically privileged tissues such as the germ cells of the testis, fetal ovary and placenta (19). The tumor cells expressing these CTAs may be recognized by autologous cytotoxic T lymphocytes, which in turn, may mediate rejection responses (20). The present study demonstrated that some CTAs, such as MAGED1 and CTAG1B, had mRNA expression in some MSCs from different fetal and adult tissues. In contrast, PRAME was expressed at very low levels compared to the controls CLs.

MAGED1, a member of the melanoma antigen family, may act as an anti-tumoral immune target (21). Also known as NRAGE, this CTA has been related with metastasis suppression of melanoma and pancreatic cancer (22) and has also been associated with the regulation of p53 transcriptional activity and the inhibition of cell proliferation (23). Another CTA, CTAG1B (also known as NY-ESO-1), has an unknown function. The exceptional immunogenicity of this CTA and its widespread distribution among several cancer types makes it an excellent target for vaccine development (24).

Cancer immunotherapy involving CTAs as targets is in development (25) and, currently, studies using CTAG1B and MAGE-A4 as targets are in clinical trials against cancers that express these antigens (26-29). However, normal tissues and MSCs of different origins may express some CTAs antigens. This expression may be explained by consistent links between normal stem cells and cancer stem cells (30, 31). Serakinci *et al.* (32) investigated the neoplastic potential of adult stem cells and suggested that MSCs may be targets for neoplastic transformation. In addition, it was demonstrated that both MAGED1 and CTAG1B are expressed in human undifferentiated MSCs from adult bone marrow and fetal liver and, after osteocyte and adipocyte differentiation, these CTAs were down-regulated (3). Joyner *et al.* (33) detected the presence of mRNA of MAGE-A4, but not the antigen, in muscle samples. In other studies, CTAG1B mRNA was also detected at low levels in normal tissues such as the pancreas, liver and breast (34, 35). The present study demonstrated that MAGE-A4 was expressed in adipocytes differentiated from

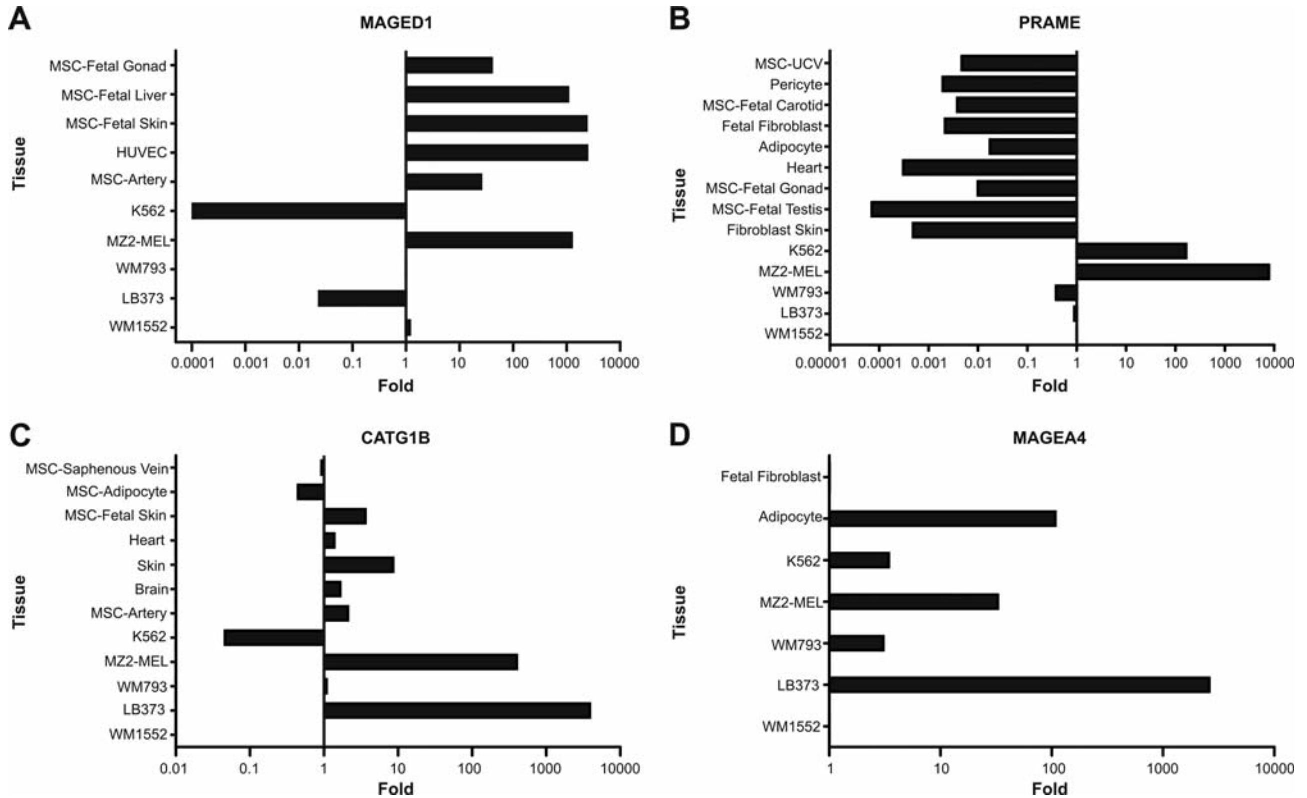

Figure 1. *CTAs mRNA expression levels of MSCs isolated from different tissues. (A) MAGED1 mRNA levels of MSCs isolated from fetal tissues (gonad, liver and skin), MSCs isolated from adult tissue (artery), HUVEC and controls. (B) PRAME mRNA levels of MSCs isolated from fetal tissues (umbilical cord vein, carotid, gonad and testis), fetal tissue cells (fibroblasts), adult tissues cells (pericytes, adipocytes, heart and fibroblasts from skin) and controls. (C) CTAG1B mRNA levels of MSCs isolated from fetal tissue (skin), MSCs isolated from adult tissues (saphenous vein, adipose and artery), adult tissues cells (heart, skin and brain) and controls. (D) MAGEA4 mRNA levels of fetal tissue (fibroblasts), adult tissue cells (adipocytes) and controls.*

MSCs and that CTAG1B was expressed in normal tissues and MSCs isolated from fetal skin, when compared to the control tumor CLs. Despite the detected expression of mRNA of some CTAs, it is not clear whether the corresponding antigen is being expressed, hampering any conclusive assumption related to potential off-target effects of eventual therapies.

PRAME is expressed in a range of carcinomas and, similar to other CTA antigens, low or no expression is observed in healthy tissue. The expression of PRAME has been linked to poor prognosis for neuroblastoma and breast cancer (36, 37), but it is associated with good prognosis in childhood acute myeloid leukemia (38). Thus, the prognostic significance of PRAME expression in malignant diseases is not clear (39), and larger studies are necessary to determine whether it is informative for prognostic purposes (38). Despite this controversy, the aberrant PRAME expression in chronic lymphocytic leukemia and mantle cell lymphoma was recently shown (40). Similarly, Greiner *et al*. (41) showed the expression of PRAME in patients with acute myeloid leukemia. In the present study, the expression of PRAME mRNA in MSCs from different tissues was demonstrated, albeit at lower levels compared to control cancer CLs. PRAME expression was also reported in CD34+ hematopoietic stem cells – (HSCs) (38). Taken together, these studies suggest that although PRAME may be an important marker for diagnosis, its potential use as a target for immunotherapy should take into account the potential off-target effects on adult stem cells such as MSCs or CD34+ HSCs.

Whether the expression of CTAs reflects cell source or a feature acquired during cell culture remains to be elucidated. Additional studies evaluating the expression of CTAs in adult stem cells, such as MSC, need to be performed in order to ascertain the possible roles of these molecules. In addition, the side effects resulting from targeting MSCs from different tissues should be considered in CTA-based immunotherapies.

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