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DNA Methylation-Associated Repression of Cancer-Germline Genes in Human Embryonic and Adult Stem Cells

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ABSTRACT

Cancer-germline (CG) genes are a particular group of germline-specific genes that rely primarily on DNA methylation for repression in somatic tissues. In a wide variety of tumors, the promoter of these genes is demethylated, and their transcription is activated. The mechanism underlying this tumor-specific activation is still unclear. It was recently suggested that CG gene expression may be a hallmark of stem cells, and that expression of these genes in several tumors may reflect the expansion of constitutively expressing cancer stem cells. To clarify this issue, we carefully evaluated the expression of several CG genes in human stem cells of embryonic and adult origin. We found no or very weak expression of CG genes in these cells. Consistently, the promoter of CG genes was highly methylated in these cells. We conclude that CG genes do not qualify as "stemness" genes, and propose that their activation in cancers results from a tumor-specific activation process. STEM CELLS *2009;27:822–824*

Disclosure of potential conflicts of interest is found at the end of this article.

A group of genes with germline-specific expression becomes activated in a wide variety of tumors. Such genes were named cancer-germline (CG) or cancer-testis (CT) genes. Importantly, CG genes encode tumor-specific antigens, which serve as targets in anticancer vaccinations trials [1]. CG genes seem to exert a variety of cellular functions, but their contribution to malignant progression is still uncertain. DNA methylation is an essential component of CG gene repression in somatic tissues, and activation in tumors has been associated with promoter hypomethylation [2].

In a recent review, Costa et al. raised the hypothesis that the expression of CG genes may be a hallmark of stem cells, and may be linked to stem cell biology [3]. Supporting evidence was provided by a previous study from the same group reporting expression of several CG genes in undifferentiated human mesenchymal stem cells (MSCs), but not in their differentiated derivatives [4]. On the basis of these findings, the authors proposed that CG gene expression in tumors may not result from a gene activation process, but may rather reflect the expansion of constitutively expressing cancer stem cells. Costa et al. further hypothesized that the expression of CG genes may be essential for embryonic development, and suggested to address this issue by studying embryonic stem (ES) cells.

In this study, we evaluated the expression level of several CG genes in human ES (HUES) cell lines. Given the strict association between CG gene activation and promoter demethylation, we also analyzed the methylation status of CG gene promoters. We tested four HUES cell lines (HUES-1, -6, -9, and -16) [5], and a human embryonal carcinoma cell line (TERA-1) [6]. Maintenance of the undifferentiated state of the cells in our culture conditions was confirmed by the high level of expression of the pluripotency marker OCT-4, which was assessed either at the mRNA level by quantitative reverse transcription polymerase chain reaction (RT-PCR; Fig. 1A), or at the protein level by immunostaining (data not shown). Quantitative RT-PCR analyses examining a total of eighteen CG genes revealed either no expression or low mRNA levels, when compared with those in expressing melanoma cells or in a testicular tissue sample (Fig. 1A). Consistently, whereas the promoters of CG genes *MAGE-A1* and *LAGE-1* were mostly unmethylated in expressing melanoma cell lines, they were highly methylated in HUES-9 and TERA-1 cells (Fig. 1B). As expected, the promoter of *OCT-4* showed the opposite methylation profile (Fig. 1B). In light of these results, we decided to re-evaluate the expression of CG genes in bone marrow MSCs. With the exception of *LAGE-1*, which showed a low but detectable expression level, all CG genes seemed to

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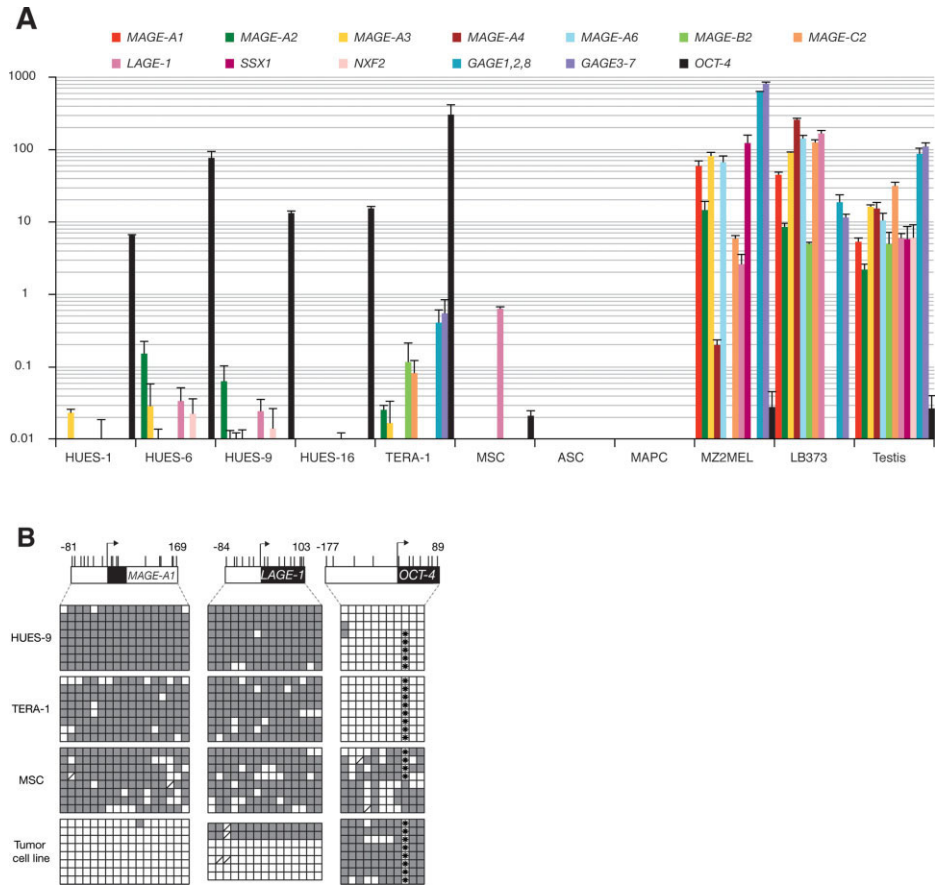

Figure 1. Expression and methylation status of cancer-germline genes and *OCT-4* in stem cells. **(A):** Real-time reverse transcription polymerase chain reaction analyses (For experimental procedures see supporting information). The expression levels are given in number of mRNA molecules/2000 *β-ACTIN* molecules. Similar results were obtained when values were normalized to glyceraldehyde-3-phosphate dehydrogenase or *RPS26* expression levels (supporting information Fig. S1). Of note, values for the *GAGE* family of genes correspond to the cumulative expression levels of the indicated genes, which were amplified indiscriminately. MZ2MEL and LB373 are two melanoma cell lines, MSCs and MAPCs are described elsewhere [7, 8], and ASCs were purchased from Zen-Bio Inc. (Research Triangle Park, NC, http://www.zen-bio.com). **(B):** Methylation patterns assessed by bisulfite sequencing (For experimental procedures see supporting information). For each gene, the region analyzed is shown: broken arrow, transcription start site; vertical bars, CpG dinucleotides; black box, exon 1. Methylation data are given below: shaded, methylated; empty, unmethylated; hatched, undetermined. Asterisks indicate polymorphism in *OCT-4* where CpG is replaced by CpA. *MAGE-A1* and *OCT-4* methylation was analyzed in MZ2MEL; *LAGE-1* methylation in LB373. Abbreviations: ASC, adipose-derived stem cells; HUES, human embryonic stem cell lines; MAPC, multipotent adult progenitor cells; MSC, mesenchymal stem cell.

be silent in MSCs (Fig. 1A). Moreover, the promoter of both *MAGE-A1* and *LAGE-1* was mostly methylated in these cells (Fig. 1B). To further assess CG gene expression in human adult stem cells, we analyzed adipose-derived stem cells (ASC) and bone marrow-derived multipotent adult progenitor cells. We did not detect any expression of CG genes in either of these cells (Fig. 1A).

In conclusion, our results do not support the notion that CG gene expression is a hallmark of stem cells. The contrasting data reported by Costa et al. probably reflects leaky repression of CG genes in these cells. Their referenced study used a highly sensitive but not quantitative nested PCR approach to detect CG gene expression in MSCs [4]. In the germ line, however, certain CG genes are possibly involved in stem cell biology, as suggested by the presence of CG proteins in the early stages of germ cell development [9]. We nevertheless conclude, at least for the CG genes analyzed in this study, that their expression in somatic tumors does not reflect the expansion of expressing precursor stem cells, but results from an epigenetic activation process that occurs during tumorigenesis.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflict of interest.

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