Cyclin D2 Promotes the Proliferation of Human Mesenchymal Stem Cells

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Abstract

Background: Human mesenchymal stem cells (hMSCs) hold promise for use in cell-based therapies and tissue engineering. Although hMSCs are thought to be stable \textit{ex vivo}, it is possible that they undergo an undesirable transformation to a phenotype of unlimited proliferation during \textit{ex vivo}. In this study, we searched for the factor required for unlimited proliferation of hMSCs.

Methods: Changes in gene expression were evaluated between hMSCs and Ewing's sarcoma cell lines, which may be derived from hMSCs, using GeneChip Human Genome U133 plus 2.0 Array. A gene up-regulated at least 10-fold in Ewing's sarcoma cell lines, Cyclin D2, was overexpressed in hMSCs by a lentiviral vector.

Results: Overexpression of Cyclin D2 in hMSCs altered cell morphology and promoted cell proliferation. Expression of transforming growth factor-\(\beta\) (TGF-\(\beta\)) (TGF-\(\beta\)-II), which induces senescence in hMSCs, was down-regulated in Cyclin D2-overexpressing hMSCs. Furthermore, Gene Ontology analysis revealed that Cyclin D2 overexpression activated expression of genes associated with proliferation and interphase.

Conclusions: Cyclin D2 promotes hMSC proliferation and is a candidate biomaker for hMSC transformation.

Keywords: hMSCs; Ewing's sarcoma; Cyclin D2; Cell proliferation

Introduction

Mesenchymal stem cells (MSCs) self-replicate and differentiate into a variety of cell types such as osteoblasts, chondrocytes, adipocytes, and smooth muscle cells [1-5]. These capacities have made MSCs useful in studies of bone and cartilage regeneration [6-8]. One of the sources of human MSCs (hMSCs) is adult bone marrow, although they occur at a rate of one per one-hundred-thousand nucleated cells [6], and the available volume of bone marrow is limited. To secure the numbers of hMSCs required for tissue regeneration, the cell must be expanded \textit{ex vivo}. Although hMSCs are stable \textit{ex vivo}, it is possible that they undergo transformation to an unlimited proliferation phenotype during expansion.

Previous studies have demonstrated that Ewing's sarcoma is derived from MSCs [9-12]. Ewing's sarcoma is a malignancy that primarily affects children and young adults, with a peak incidence between the ages of 14 and 20 years. It arises mainly in bone and less commonly in soft tissues. The t(11;22)(q24;q12) chromosomal translocation generating EWS-FLI-1 fusion gene is found in 85% of cases [13]. EWS-FLI-1 knockdown inhibits cell proliferation in Ewing's sarcoma cells [14,15]. Thus, EWS-FLI-1 expression does not transform normal murine and human fibroblasts [16,17], suggesting EWS-FLI-1 promotes malignant transformation in selective cells.

Several reports have demonstrated that EWS-FLI-1 expression transforms murine MSCs; indeed, tumors form when these cells are injected into immunodeficient mice [9,12]. In contrast, EWS-FLI-1 expression in hMSCs does not accelerate cell proliferation and transformation (10). EWS-FLI-1 expression in hMSCs induces a gene expression profile that closely mimics that of Ewing's sarcoma [9-11] without affecting proliferation. Therefore, MSCs are thought to be the origin of Ewing's sarcoma, but because EWS-FLI-1 alone cannot transform hMSCs, we believe other factors are required for transformation.

The most important safety concern when using hMSCs in cell-based therapies and tissue engineering is the occurrence of unlimited proliferation during \textit{ex vivo} culture. To identify the factors required for unlimited hMSC proliferation, we compared the gene expression profiles of hMSCs and Ewing's sarcoma cell lines and found that Cyclin D2 expression was extremely high in the Ewing's sarcoma cell lines. Overexpression of Cyclin D2 promotes proliferation of hMSCs, suggesting that Cyclin D2 is a candidate biomaker for hMSC transformation.

Materials and Methods

Cell culture

hMSCs derived from bone marrow were purchased from Lonza (Walkersville, MD) and cultured in MSCGM BulletKit, a mesenchymal stem cell basal medium with mesenchymal cell growth supplement, L-glutamine, and gentamicin/amphotericin-B (Lonza Walkersville, MD). Ewing's sarcoma cell lines (Hs 822.T, Hs 863.T, RD-ES, and SK-ES-1) were purchased from American Type Culture Collection (ATCC; Manassas, VA). Hs 822.T and Hs 863.T were cultured in Dulbecco's Modified Eagle's medium (DMEM; Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco). RD-ES was cultured in RPMI-1640 medium (Gibco) supplemented with 10% FBS. SK-ES-1 was cultured in McCoy's 5a medium modified (Gibco) supplemented with 15% FBS. 293T (human kidney; ATCC) was cultured in DMEM supplemented with 10% FBS.

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Received October 24, 2013; Accepted December 26, 2013; Published December 28, 2013

Citation: Kono K, Niimi S, Sawada R (2013) Cyclin D2 Promotes the Proliferation of Human Mesenchymal Stem Cells. J Bone Marrow Res 2: 136. doi: 10.4172/2329-8820.1000136

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Microarray analysis

Total RNA was extracted from hMSCs and Ewing’s sarcoma cell lines with the RNeasy Mini Kit (QIAGEN, Valencia, CA). Total RNA quantity and quality were assessed on an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA); 100 ng total RNA was used to generate biotin-modified amplified RNA (aRNA) with GeneChip 3′IVT Express Kit (Affymetrix, Santa Clara, CA). Reverse transcription (RT) of first-strand complementary DNA (cDNA) with the T7 promoter sequence was performed with the T7 oligo(dT) primer. Second-strand cDNA synthesis was used to convert the single-stranded cDNA into a double-stranded DNA template by using DNA polymerase and RNase H to simultaneously degrade the RNA and synthesize second-strand cDNA. In vitro transcription of biotin-modified aRNA with IVT Labeling Master Mix generated multiple copies of biotin-modified aRNA from the double-stranded cDNA templates. The aRNA was purified and quantified; after fragmentation, it was hybridized to GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix). The arrays were stained with phycoerythrin and washed at the GeneChip Fluidics station 450 (Affymetrix). The microarrays were scanned and data extracted using GeneChip scanner 3000 7G (Affymetrix); image analysis was performed using the Affymetrix GeneChip Command Console Software and digitized with the Affymetrix Expression Console.

Data processing and pathway analysis

Data analysis was performed with GeneSpring GX 11.0 software (Agilent Technologies, Santa Clara, CA). Raw data were normalized to the 50th percentile per chip and the median per gene. Differentially expressed genes were analyzed using Ingenuity Pathway Analysis (IPA) 9.0 (Ingenuity Systems, Redwood City, CA). Fisher’s exact test was used to calculate a P-value. Activation z-score was calculated as a measure of functional and translational activation in Networks and Upstream regulators analysis. An absolute z-score >2 was considered significant.

Real-time RT-PCR

Total RNA was reverse-transcribed with SuperScript III First-Strand Synthesis System for RT-PCR (Life Technologies Co., Carlsbad, CA). Real-time RT-PCR was performed with LightCycler Fast Start DNA Master SYBR Green I (Roche, Basel, Switzerland) in a Roche LightCycler instrument (software version 4.0). mRNA expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers for Cyclin D2 and p16 were 5′-TACTTCAAGTGCGTGCAGAAGAC-3′ and 5′-TCCCA-CACCTCCAGTGGCGATCAT-3′ (Cyclin D2) and 5′-CCTCAGC-GCCCTAAAGC-3′ and 5′-GCAGTGTGACTCAAGAGAA-3′ (p16). The primers for transforming growth factor-β2 (TGF-β2) and GAPDH were from Light Cycler Primer Sets (Search LC GmbH, Heidelberg, Germany).

Cloning and expression of Cyclin D2

Cyclin D2 cDNA was amplified by RT-PCR of mRNA extracted from SK-ES-1 using 5′-GAATTCCGCGCCTATAGGCTGCTGTT-GCCAGGAG-3′ (forward: EcoR I site underlined) and 5′-CTCGAGTCACAGGTGCATATCTGGCGACG-3′ (reverse; Xho I site underlined). The amplified products were cloned into pT2A (ToYoBo, Osaka, Japan) and verified by sequencing. The verified Cyclin D2 cDNA was cloned into the EcoR I and Xho I sites of pLVsIN-CMV Pur (TaKaRa, Shiga, Japan). Lentiviral vector was prepared with the Lenti-XTM Packaging System (TaKaRa) according to manufacturer protocols.

Viral infection

hMSCs were infected with the lentiviral vector containing Cyclin D2 (hMSCs/CyclinD2) or empty vector (hMSCs/Empty) at 37°C for 24 h. Infected cells were selected with 1 μg/mL puromycin for 14 days and the bulk of the resistant cells was used in subsequent experiments.

Western blotting

hMSCs/CyclinD2 and hMSCs/Empty were lysed in RIPA buffer (Wako, Osaka, Japan). Cyclin D2 was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a membrane (Immunobilon-P, Millipore, Billerica, MA). Blots were blocked and probed overnight at 4°C with a mouse monoclonal antibody against Cyclin D2 (MBL, Nagoya, Japan). Blots were incubated with peroxidase-conjugated anti-mouse IgG (Abcam) and bound antibodies were visualized with Chemi-Lumi One Super Chemiluminescence (Nacalai Tesque, Kyoto, Japan).

Cell proliferation

Proliferation of hMSCs/CyclinD2 and hMSCs/Empty was measured with the TetraColor ONE reagent (Seikagaku Co., Tokyo, Japan). Cultures were incubated for 2 h in medium containing the reagent. Absorbance was read at 450 nm (reference at 600 nm) on a plate reader (SH-9000, Corona Electric Co., Ibaraki, Japan).

Results

Cyclin D2 expression in Ewing’s sarcoma cell lines versus hMSCs

To identify the factors required for proliferation of hMSCs, we compared the gene expression profiles of hMSCs and four Ewing’s sarcoma cell lines (Hs 822.T, Hs 863.T, RD-ES, and SK-ES-1) (Figure 1a), Hs 822.T and Hs 863.T had similar expression profiles, as did RD-ES and SK-ES-1. The expression profiles of Hs 822.T and Hs 863.T were more similar to those of hMSCs than to those of the other Ewing’s sarcoma (RD-ES and SK-ES-1). Therefore, we first compared the expression profiles of hMSCs, Hs 822.T, and Hs 863.T. We identified 44 genes that differed by at least 10-fold between hMSCs and Ewing’s sarcoma cell lines (data not shown). These were narrowed to 9 genes by selecting genes that also differed from hMSCs by more than 10-fold in RD-ES and SK-ES-1 (Table 1). CCND2 (Cyclin D2) stood out in this group of 9 genes, because it represents a family of key cell-cycle regulators. Indeed, aberrant expression of
Cyclin D2 has been associated with tumor progression in many tumor types [18-21]. We measured Cyclin D2 mRNA expression in hMSCs and Ewing's sarcoma cell lines by real-time PCR and confirmed its extreme induction in Ewing's sarcoma (Figure 1b). Hs 822.T and Hs 863.T exhibited similar Cyclin D2 expression levels; expression was higher in RD-ES and SK-ES-1.

**Overexpression of Cyclin D2 promoted hMSC proliferation**

We transduced the Cyclin D2 gene into hMSCs by using a lentiviral vector; an empty vector served as a negative control. At 14 days after infection when puromycin selection was completed, Cyclin D2 mRNA expression in hMSCs/CyclinD2 was about 10,000-fold higher than in hMSCs infected with empty vector (hMSCs/Empty) (Figure 2a). Cyclin D2 was stably expressed in hMSCs at least 56 days after infection (Figure 2b). Next, we observed the cell morphology of the hMSCs/CyclinD2. Phase contrast microscopy revealed the normal fibroblast-like morphology of hMSCs/Empty (Figure 2c) and smaller spread areas in hMSCs/CyclinD2 (Figures 2c and 2d). Furthermore, proliferation of hMSCs/CyclinD2 was faster than that of hMSCs/Empty, indicating that overexpression of Cyclin D2 promoted hMSC proliferation. However, the proliferation slowed over time and did not result in unlimited proliferation (data not shown).

**TGF-β2 expression was down-regulated in hMSCs/CyclinD2, but p16 expression was not**

To investigate the effects of overexpression of Cyclin D2 on the cell cycle, we examined the change in cell cycle-associated gene expression over time (p16, p21, Bmi1, TGF-β1, and TGF-β2). We did not detect significant differences in expression of p21, Bmi1, and TGF-β1 between hMSCs/CyclinD2 and hMSCs/Empty (data not shown). However, TGF-β2 expression was lower in hMSCs/CyclinD2 than in hMSCs/Empty (Figure 3a). In addition, the increase in TGF-β2 expression during culture was suppressed in hMSCs/CyclinD2 compared with hMSCs/Empty. In contrast, the increasing rate of p16 expression in hMSCs/CyclinD2 was higher than in hMSCs/Empty, although expression in both cell types was comparable 14 days after infection (Figure 3b).

**Overexpression of Cyclin D2 altered the expression of genes associated with cell proliferation and interphase**

Total RNA was extracted from hMSCs/CyclinD2 and hMSCs/Empty 14 days after infection and analyzed by DNA microarray, which identified 690 genes that were differentially expressed by at least 2-fold between hMSCs/CyclinD2 and hMSCs/Empty.
(Figure 4a). Gene ontology (GO) analysis revealed these genes are associated with movement, development, growth and proliferation, cell cycle, and intercellular signaling and interactions (Table 2). Specific predictions indicated that proliferation and interphase are activated in hMSCs/CyclinD2. The induced genes that are associated with proliferation and interphase are listed in tables 3 and 4; in summary, 94 of 186 genes and 19 of 50 genes exhibited expression shifts consistent with increased in proliferation and interphase, respectively.

Discussion

Although EWS-FLI-1 expression transformed murine MSCs, expression in hMSCs did not promote cell proliferation. In this study, we found that Cyclin D2 expression was extremely high in the Ewing’s sarcoma cell lines and overexpression of Cyclin D2 in hMSCs promoted cell proliferation. GO analysis also predicted that cell proliferation and interphase were activated by overexpression of Cyclin D2.

Cyclin D2 is a member of the family of D-type cyclins that mediate cell cycle regulation, differentiation, and oncogenic transformation [22,23]. D-type cyclins inactivate retinoblastoma (Rb) by phosphorylation, inducing release of E2F. Free E2F activates genes involved in the activation and maintenance of DNA synthesis. Thus, overexpression of Cyclin D2 generally has growth-promoting effects. Consistent with this notion, overexpression of Cyclin D2 in HeLa
Table 2: Global gene expression in hMSCs/CyclinD2. Gene ontology (GO) analysis of the 690 genes was performed with Ingenuity Pathway Analysis (IPA) 9.0. Top five functional categories and the specified categories are listed. An absolute z-score >2 was considered as significant.

| ID   | Genes in dataset | Fold Change | Prediction (based on expression direction) | ID   | Genes in dataset | Fold Change | Prediction (based on expression direction) | ID   | Genes in dataset | Fold Change | Prediction (based on Expression direction) |
|------|------------------|-------------|--------------------------------------------|------|------------------|-------------|--------------------------------------------|------|------------------|-------------|--------------------------------------------|
| 209292_at | ID4  | 3.609 | Increased | 201195_s_at | SLC7A5 | 2.083 | direction) | 203083_at | THBS2 | 2.096 | Decreased |
| 206271_at | TLR3  | 2.023 | Increased | 222749_at | SDF1 | -3.251 | Increased | 203468_at | CDK10 | 2.228 | Increased |
| 203373_at | SOCS2 | 3.513 | Increased | 204050_s_at | CA12 | 3.237 | Increased | 1552721_a_at | FGF1 | -2.054 | Decreased |
| 206649_s_at | TFE3 | 2.069 | Increased | 203764_at | DLGAP5 | 2.188 | Increased | 204052_s_at | SFRP4 | 2.249 | Increased |
| 201292_at | TOP2A | 2.434 | Increased | 214012_at | ERAP1 | 5.288 | Increased | 205548_s_at | BTG3 | 2.073 | Increased |
| 204766_s_at | NUDT1 | 2.158 | Increased | 204614_at | SERPINB2 | -2.048 | Increased | 242869_at | STRN | 2.145 | Increased |
| 209321_s_at | ADCY3 | 2.329 | Increased | 201416_at | SOX4 | 3.11 | Increased | 213905_x_at | BGN | 2.09 | Increased |
| 206693_at | IL7 | 2.399 | Increased | 214581_x_at | TNFRSF21 | -2.049 | Increased | 228780_at | Pou3F3 | -2.721 | Decreased |
| 205345_at | BARD1 | 2.029 | Increased | 223570_s_at | MCM10 | 2.545 | Increased | 205016_at | TGFα | -3.032 | Decreased |
| 226377_at | NFIC | 2.026 | Increased | 219743_at | HEY2 | 2.13 | Increased | 1569791_at | STK4 | 2.396 | Increased |
| 212148_at | PBX1 | 2.514 | Increased | 209970_at | GAS2 | 3.144 | Increased | 216008_s_at | ARHI | 2.331 | Increased |
| 20223_s_at | CUL4B | 2.093 | Increased | 202684_s_at | RNF142 | 3.237 | Increased | 221577_x_at | GDF5 | 2.143 | Increased |
| 224954_s_at | SHMT1 | 2.105 | Increased | 242979_at | IR51 | 2.329 | Increased | 202153_s_at | NUP2 | 2.09 | Increased |
| 209960_at | HGF | 2.732 | Increased | 225141_at | NFATC3 | 2.553 | Increased | 202556_s_at | MCR5 | 2.165 | Increased |
| 217371_at | IL15 | 2.953 | Increased | 218030_at | GIT1 | 3.302 | Increased | 203395_at | HEH | 2.898 | Increased |
| 205887_s_at | MSH3 | 2.1 | Increased | 218750_at | TAF1D | 2.304 | Increased | 203184_at | FBNU | 2.145 | Increased |
| 1568685_s_at | FNTB | 2.556 | Increased | 201795_at | LBR | 2.383 | Increased | 203904_s_at | CDB2 | 2.382 | Increased |
| 208296_s_at | TNFAP8 | 2.118 | Increased | 210045_at | IDH2 | 2.018 | Increased | 206558_at | SIM2 | 2.745 | Increased |
| 226534_s_at | KITLG | 2.22 | Increased | 1553810_at | KIAA1524 | 2.457 | Increased | 203665_at | HMOX1 | 2.358 | Increased |
| 214981_at | POSTN | 2.095 | Increased | 209190_at | AKR1C3 | 2.281 | Increased | 203543_s_at | KLF9 | 2.577 | Increased |
| 206026_s_at | TNFAIP6 | 2.008 | Increased | 233424_at | PRDM16 | 2.334 | Increased | 1557729_at | GRK5 | 2.098 | Increased |
| 210135_s_at | SHOX2 | 2.194 | Increased | 1554509_s_at | FAM188A | -2.494 | Increased | 236028_at | IBSP | -3.459 | Decreased |
| 204457_s_at | GAS1 | 2.134 | Increased | 217949_at | PTENP1 | -2.849 | Increased | 221539_at | E14BP1 | 2.487 | Increased |
| 221884_s_at | MECOM | 2.004 | Increased | 216205_s_at | MIFN2 | -2.34 | Increased | 202430_s_at | PLSCR1 | 2.324 | Increased |
| 209919_at | GGT1 | 2.63 | Increased | 200644_at | MARCKSL1 | 3.121 | Increased | 204054_at | PTEN | 2.145 | Increased |
| 229468_s_at | CDK3 | 2.022 | Increased | 217991_x_at | SSBP3 | 3.21 | Increased | 209617_at | CTNND2 | -2.01 | Decreased |
| 1563182_at | ACVR1C | -2.241 | Decreased | 211653_x_at | AKR1C1/ AKR1C2 | 2.791 | Increased | 231697_s_at | VMP1 | 2.741 | Increased |
| 227404_s_at | EGR1 | 2.543 | Increased | 228302_x_at | CAMK2N1 | -3.3 | Increased | 210143_at | ANXA10 | 2.124 | Increased |
| 210933_s_at | FSCN1 | 2.227 | Increased | 234040_at | HELLs | 2.476 | Increased | 206233_at | B4GALT6 | -2.767 | Decreased |
| 205357_s_at | AGTR1 | 2.987 | Increased | 218413_s_at | ZNF639 | 2.019 | Increased | 209705_at | MTF2 | 2.504 | Increased |
| 209925_s_at | OCLN | -2.024 | Decreased | 214970_s_at | GBP2 | 2.488 | Increased | 208802_at | PHLD2A | 2.17 | Increased |
| 205732_s_at | NCOA2 | 2.484 | Increased | 219377_at | GAREM | 2.237 | Increased | 219868_at | TMEG25 | -2.404 | Decreased |
| 215404_s_at | FGFR1 | 2.421 | Increased | 212865_s_at | COL14A1 | 2.516 | Increased | 219047_s_at | ZNF668 | 2.244 | Increased |
| 235521_at | HOXA3 | 2.05 | Increased | 203572_s_at | TAF6 | 2.79 | Decreased | 64474_g_at | DGC8 | 2.201 | Increased |
| 218807_at | VAV3 | 2.555 | Increased | 232231_at | RUNX2 | 2.12 | Decreased | 205159_at | CSF2RB | -4.605 | Decreased |
| 202202_at | LAMA4 | 3.771 | Increased | 202931_x_at | BIN1 | 2.056 | Decreased | 236012_at | PSMF1 | 2.172 | Affected |

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205500_at C5 2.375 Increased 214433_s_at SELENBP1 2.103 Decreased 235833_at PPAT 3.266 Affected
204128_s_at RFC3 2.06 Increased 228766_at CD36 -4.325 Decreased 221261_x_at MAGED4/MAGED4B 3.294 Affected
200951_at CCND2 18.529 Increased 235300_x_at RCHY1 2.569 Decreased 204983_s_at GPC4 -2.902 Affected
222073_at COLA4A3 -2.278 Increased 222999_s_at CCNL2 2.019 Decreased 231837_at USP28 2.365 Affected
226731_at ITGA1 2.816 Increased 208791_at CLU -2.242 Decreased 1554606_at CEP120 2.141 Affected
227125_at IFNAR2 2.053 Increased 203973_s_at CEBPD 2.099 Decreased 224325_at FZD8 2.488 Affected
222036_at MCM4 2.31 Increased 209383_at DDIT3 2.065 Decreased 217650_x_at ST3GAL2 2.003 Affected
204061_at PKX 2.226 Increased 219266_at ZNF350 2.143 Decreased 224022_x_at WNT16 -3.268 Affected
225962_at PDE4D 2.562 Increased 206825_at OXTR -2.396 Decreased 220962_s_at FADS1 3.311 Affected
212672_at ATM 2.024 Increased 205027_s_at MAP3K9 2.11 Decreased 202948_at IL1R1 2.512 Affected
225572_at CREB1 2.243 Increased 205891_at ADORA2B 2.166 Decreased 50277_at GGA1 2.292 Affected
203046_at TIMELESS 2.156 Increased 231947_at MYCT1 -2.144 Decreased 1555843_at HNRNPM 2.611 Affected
213943_at TWIST1 2.021 Increased 212430_at RBM38 2.254 Decreased 214157_at GNAS -2.307 Affected
209465_x_at PTN 2.357 Increased 204159_at CDKN2C 2.004 Decreased 215987_at RAPGEF2 2.046 Affected
213506_at F2RL1 4.829 Increased 212401_s_at CDK11A/CDK11B 2.086 Decreased 216237_s_at MCM5 2.753 Affected
231559_at NNMT 2.208 Increased 205080_at RARB 2.058 Decreased 206086_x_at HFE 2.218 Affected
225740_x_at MDM4 2.283 Increased 214727_at BRCA2 2.489 Decreased 229807_s_at MAZ 2 Affected
226636_at PLD1 2.022 Increased 202718_at IGFBP2 -2.099 Decreased 231002_s_at RABEP1 2.266 Affected
227048_at LAMA1 2.296 Increased 213811_x_at TCF3 2.395 Decreased 209753_s_at TMPO 2.116 Affected
230462_at NUMB 2.763 Increased 202528_at SMAD4 2.16 Decreased 201627_s_at INSIG1 3.156 Affected
201727_s_at ELAVL1 2.216 Increased 1567013_s_at NFE2L2 3.005 Decreased 218019_s_at PDK1 2.152 Affected
205204_at NMB 2.09 Increased 234339_s_at GLTSCR2 2.573 Decreased 204630_at ADA 2.051 Affected
232044_at RBBP6 2.324 Increased 206332_s_at IFI16 2.24 Decreased 236223_s_at RIT1 2.823 Affected
205394_at CHEK1 2.071 Increased 228967_at EIF1 2.161 Increased 208913_s_at GGA2 2.062 Affected
57532_at DVL2 2.03 Increased 235593_at ZEB2 2.106 Decreased 201286_at SDC1 2.164 Affected
209837_s_at ID1 2.256 Increased 156583_s_at SLC9A1 -2.156 Decreased 201106_at GPX4 2.087 Affected

'Increased' means the genes up- or down-expression is predicted to promote proliferation of cells. 'Decreased' means the up- or down-expression is predicted to inhibit. 'Affected' means IPA could not predict whether the expression change promote or inhibit.

Table 3: 'Proliferation of cells' genes differentially expressed by at least 2-fold between hMSCs/CyclinD2 and hMSCs/Empty.
was aberrantly expressed in Ewing's sarcoma (Table 1), consistent of hMSCs but did not lead to unlimited proliferation. Other factors probably prevented unlimited proliferation. Consistent with this notion, some Ewing's sarcomas contain a homozygous deletion of the p16 locus [16], possibly facilitating subsequent transformation.

In this study, overexpression of Cyclin D2 promoted proliferation of hMSCs but did not lead to unlimited proliferation. Other factors are required for the unlimited proliferation of hMSCs. IGF2BP1 was aberrantly expressed in Ewing's sarcoma (Table 1), consistent with a previous report of an association between increased IGF2BP1 expression and tumor progression in patients with lung cancer [30]. Thus, we attempted to transduce the IGF2BP1 gene into hMSCs, but IGF2BP1 expression was up-regulated by only 2-fold and transduction efficiency was low (data not shown). The cause for this inefficiency is unclear. Because the growth kinetics of IGF2BP1-transformed E. coli is quite slow (data not shown), it is likely that overexpression of IGF2BP1 is deleterious for hMSCs.

We did not tested whether the other genes listed in Table 1 affect proliferation of hMSCs, because these genes were not thought to directly affect the proliferation. Furthermore, not all Ewing’s sarcomas express EWS-FLI-1: indeed, EWS-FLI-1 mRNA was not detected in Hs 822.T and Hs 863.T (data not shown). Thus, we did not transduce the EWS-FLI-1 gene into hMSCs. However, it is possible that the cooperation of these proteins is important for the development of Ewing’s sarcoma. Thus, it would be interesting to transduce these genes into hMSCs in addition to Cyclin D2.

Conclusion

Cyclin D2 promotes hMSC proliferation and is a candidate biomaker for hMSC transformation.

Acknowledgements

The authors would like to thank Atsuko Matsuoka for helpful discussions. This work was supported by the Health and Labor Sciences Research Grants for Research on Regulatory Science of Pharmaceuticals and Medical Devices (H23-IYAKU-SHITEI-027) from the Ministry of Health, Labor and Welfare of Japan.

References

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, et al. (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284: 143-147.
2. Caplan AI, Bruder SP (2001) Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. Trends Mol Med 7: 259-264.

3. Gojo S, Gojo N, Takeda Y, Mori T, Abe H, et al. (2003) In vivo cardiovasculogenesis by direct injection of isolated adult mesenchymal stem cells. Exp Cell Res 288: 51-59.

4. Wakiyani S, Saito T, Caplan AI (1995) Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. Muscle Nerve 18: 1417-1426.

5. Prockop DJ (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276: 71-74.

6. Ohgushi H, Caplan AI (1999) Stem cell technology and bioceramics: from cell to gene engineering. J Biomed Mater Res 48: 913-927.

7. Petite H, Viateau V, Bensaid W, Meunier A, de Pollak C, et al. (2000) Tissue-engineered bone regeneration. Nat Biotechnol 18: 959-963.

8. Ochi M, Adachi N, Nobuto H, Yanada S, Ito Y, et al. (2004) Articular cartilage repair using tissue engineering technique-novel approach with minimally invasive procedure. Artif Organs 28: 28-32.

9. Riggi N, Cirioni L, Provero P, Suvá ML, Kaloulis K, et al. (2005) Development of Ewing’s sarcoma from primary bone marrow-derived mesenchymal progenitor cells. Cancer Res 65: 11459-11469.

10. Riggi N, Suvá ML, Suvá D, Cirioni L, Provero P, et al. (2008) EWS-FLI-1 expression triggers a Ewing’s sarcoma initiation program in primary human mesenchymal stem cells. Cancer Res 68: 2176-2185.

11. Miyagawa Y, Okita H, Nakajima H, Horiiuchi Y, Sato B, et al. (2008) Inducible expression of chimeric EWS/ETS proteins confers Ewing’s family tumor-like phenotypes to human mesenchymal progenitor cells. Mol Cell Biol 28: 2125-2137.

12. Castillero-Trejo Y, Eliazer S, Xiang L, Richardson JA, Ilaria RL Jr (1997) Expression of the EWS/FLI-1 oncogene in murine primary bone-derived cells Results in EWS-FLI-1-dependent, ewing sarcoma-like tumors. Cancer Res 57: 8698-8705.

13. Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, et al. (1992) Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature 359: 162-165.

14. Toretsky JA, Connell Y, Neckers L, Bhat NK (1997) Inhibition of EWS-FLI-1 fusion protein with antisense oligodeoxynucleotides. J Neurooncol 31: 9-16.

15. Tanaka K, Ikawakuma T, Harimaya K, Sato H, Iwamoto Y (1997) EWS-FLI1 antisense oligodeoxynucleotide inhibits proliferation of human Ewing’s sarcoma and primitive neuroectodermal tumor cells. J Clin Invest 99: 239-247.

16. Deneen B, Denny CT (2001) Loss of p16 pathways stabilizes EWS/FLI1 expression and complements EWS/FLI1 mediated transformation. Oncogene 20: 6731-6741.

17. Lessnick SL, Dacwag CS, Golub TR (2002) The Ewing’s sarcoma oncoprotein EWS/FLI induces a p53-dependent growth arrest in primary human fibroblasts. Cancer Cell 1: 393-401.

18. Takano Y, Kato Y, Masuda M, Ohshima Y, Okayasu I (1999) Cyclin D2, but not cyclin D1, overexpression closely correlates with gastric cancer progression and prognosis. J Pathol 189: 194-200.

19. Takano Y, Kato Y, van Diest PJ, Masuda M, Mitomi H, et al. (2000) Cyclin D2 overexpression and lack of p27 correlates positively and cyclin E inversely with a poor prognosis in gastric cancer cases. Am J Pathol 156: 585-594.

20. Merrelsstein A, Gerson A, Walfisch S, Delgado B, Shechter-Maor G, et al. (2005) Expression of D-type cyclins in colon cancer and in cell lines from colon carcinomas. Br J Cancer 93: 338-345.

21. Dhillon VS, Shahid M, Haisun SA (2004) CpG methylation of the Fhit, FANC, cyclin-D2, BRCA2 and RUNX3 genes in Granulosa cell tumors (GCTs) of ovarian origin. Mol Cancer 3: 33.

22. Sherr CJ (1993) Mammalian G1 cyclins. Cell 73: 1059-1065.

23. Bartkova J, Rajpert-de Meyts E, Skakkebaek NE, Bartek J (1999) D-type cyclins in adult human testis and testicular cancer: relation to cell type, proliferation, differentiation, and malignancy. J Pathol 187: 573-581.

24. Goodwin EC, DiMaio D (2000) Repression of human papillomavirus oncogenes in HeLa cervical carcinoma cells causes the orderly reactivation of dormant tumor suppressor pathways. Proc Natl Acad Sci U S A 97: 12513-12518.

25. Meyyappan M, Wong H, Hull C, Riabowol KT (1998) Increased expression of cyclin D2 during multiple states of growth arrest in primary and established cells. Mol Cell Biol 18: 3163-3172.

26. Sawada R, Ito T, Tsuchiya T (2006) Changes in expression of genes related to cell proliferation in human mesenchymal stem cells during in vitro culture in comparison with cancer cells. J Artif Organs 9: 179-184.

27. Ito T, Sawada R, Fujiwara Y, Seyama Y, Tsuchiya T (2007) FGFS suppresses cellular senescence of human mesenchymal stem cells by down-regulation of TGF-beta2. Biochem Biophys Res Commun 359: 108-114.

28. Krishnamurthy J, Ramsey MR, Ligon KL, Torrice C, Koh A, et al. (2006) p16INK4a induces an age-dependent decline in islet regenerative potential. Nature 443: 451-457.

29. Liggett WH Jr, Sidransky D (1998) Role of the p16 tumor suppressor gene in cancer. J Clin Oncol 16: 1197-1206.

30. Kato T, Hayama S, Yamabuki T, Ishikawa N, Miyamoto M, et al. (2007) Increased expression of insulin-like growth factor-II messenger RNA-binding protein 1 is associated with tumor progression in patients with lung cancer. Clin Cancer Res 13: 434-442.