Chromosomal instability in human mesenchymal stem cells immortalized with human papilloma virus E6, E7, and hTERT genes

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Fig. 2 FISH analysis of human mesenchymal stem cell (hMSC) lines immortalized with hTERT alone, hTERT plus bm-1, HPVE6 or with hTERT plus HPVE6/E7. Multi color FISH images of metaphase spreads (*A, D*), their karyotypes (*B, E*) and painting FISH images using DNA probes specific for chromosome 13 (*green*) and 17 (*red*)(*C, F*) of UCBTERT-21 (*A, B, C*) and UBE6T-6 (*D, E, F*). Quantity of chromo-

some 13 copy numbers in four cell lines (*G – J*). FISH signals were counted in 120~200 metaphase spreads plus interphase nuclei. UCBTERT-21 cells contained two copies of chromosome 13 and 17, and showed normal human karyotype, whereas other cells lost one copy of chromosome 13.

Fig. 3 Array CGH profiles performed on four immortalized human mesenchymal stem cell lines at selected PDL. For each panel, the X-axis represents the 22 autosomes, the X and Y chromosomes, and the Y-axis shows the Log_2 of the fluorescence intensity ratio (cy3 [hMSCs] / cy5 [normal cell]) of all spots of the chromosome. Values above 0 (red spots) or values below 0 (blue spots) signify a loss of chromosome

(chromosome regions). Blue spots in each panel indicate the Log_2 ratios observed at early stage in the culture of each cell line, which are overlaid with red spots indicated at the late stage. Green spots indicate the difference in value between blue spots and red spots. Note that in the UEGE7T-3 cell line, one copy of chromosome 13 and 16 were lost between PDL 78 and 101.

Fig. 4 Differentiation potential of immortalized human mesenchymal stem cell lines into adipogenic, osteogenic and neurogenic lineages. Adipogenesis was indicated by the accumulation of lipid stained with Oil Red-O (*Aa* and *Ab*, UE6E7T-3 cell line). Osteogenesis is indicated by the increase in alkaline phosphatase (*Ac* and *Ad*, UCB408E6E7TERT-33 cell line). Neurogenesis was shown by staining with two kinds of monoclonal antibodies to III β -tubulin and neurofilament, and by shape

changes of cell (*Ae* – *Ah*, UBE6T-6 cell line). *B*, Comparison of the differentiation potential of four cell lines whose responses to stimuli into differentiation were diverse among the cell lines. – and + indicate a response similar to an untreated cell and a weak positive response. +++ indicates a strong response shown by images of treated cells in Fig. 4*A*. (Bar indicates 20 μ m).