Cellular differentiation hierarchies in normal and culture-adapted human embryonic stem cells

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Human embryonic stem cell (HESC) lines vary in their characteristics and behaviour not only because they are derived from genetically outbred populations, but also because they may undergo progressive adaptation upon long-term culture in vitro. Such adaptation may reflect selection of variants with altered propensity for survival and retention of an undifferentiated phenotype. Elucidating the mechanisms involved will be important for understanding normal self-renewal and commitment to differentiation and for validating the safety of HESC-based therapy. We have investigated this process of adaptation at the cellular and molecular levels through a comparison of early passage (normal) and late passage (adapted) sublines of a single HESC line, H7. To account for spontaneous differentiation that occurs in HESC cultures, we sorted cells for SSEA3, which marks undifferentiated HESC. We show that the gene expression programmes of the adapted cells partially reflected their aberrant karyotype, but also resulted from a failure in X-inactivation, emphasizing the importance in adaptation of karyotypically silent epigenetic changes. On the basis of growth potential, ability to re-initiate ES cultures and global transcription profiles, we propose a cellular differentiation hierarchy for maintenance cultures of HESC: normal SSEA3+ cells represent pluripotent stem cells. Normal SSEA3– cells have exited this compartment, but retain multilineage differentiation potential. However, adapted SSEA3+ and SSEA3– cells co-segregate within the stem cell territory, implying that adaptation reflects an alteration in the balance between self-renewal and differentiation. As this balance is also an essential feature of cancer, the mechanisms of culture adaptation may mirror those of oncogenesis and tumour progression.

INTRODUCTION

Cell identity and potency is ultimately a function of gene expression. Several studies have sought to gain insights into the mechanisms of self-renewal and differentiation in human embryonic stem cells (HESCs) through global gene expression profiling of the undifferentiated stem cells and comparison with their differentiated derivatives, the latter often in the form of haphazardly differentiated embryoid bodies (1–5). Others have attempted to analyse the transcriptome by quantifying the relative abundance of expressed RNA in undifferentiated HESC and to identify those genes thought to be involved in pluripotency (6,7). A number of specific genes that are characteristically expressed in undifferentiated HESC, and downregulated upon their differentiation, have been identified in each of these studies, notably POU5F1

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