RNA interference mediated knockdown of Oct4 in human embryonal carcinoma (EC) and embryonic stem (ES) cells

Summary

Human embryonal carcinoma (EC) cells are the stem cells of teratocarcinomas, and they are key components of germ cell tumours (GCTs). Since differentiation often appears to result in loss of a malignant phenotype, perhaps genetic variants that cause loss of pluripotency have a selective advantage for tumour growth and therefore many EC cells are apparently nullipotent, ie have lost their capacity for differentiation.

Human embryonic stem (hES) cells are pluripotent cells derived from inner cell mass (ICM) of human embryos at blastocyst stage. These cells can be induced to differentiate into all three embryonic germ layers. Human ES cells offer the opportunity for the *in vitro* production of multiple cell types for use in regenerative medicine. A key to unlocking this potential is development of methods for controlling gene expression and, consequently, cell differentiation. One tool that might be exploited is RNA interference (RNAi) to manipulate specific signalling pathways in a transient manner and so influence the selection of specific pathways of differentiation by a pluripotent stem cell. RNAi is a process in which short interfering RNAs are used to silence gene expression in a sequence-specific manner and was first described by Andrew Fire and Craig Mello.

To explore this possibility we used RNAi to determine whether the transcription factor, OCT4, is required to maintain the undifferentiated state of hES cell, as well as hEC cells and whether forced knockdown of OCT4 expression results in differentiation towards trophectoderm. Murine ES cells have been shown to be dependent upon the correct levels of Oct4 expression for their maintenance of an undifferentiated stem cell phenotype, and they differentiate to trophectoderm in its absence. However, various differences exist between mouse and human ES cells (e.g. the non-responsiveness of hES cells to LIF, and their different surface antigen

phenotypes), so that one cannot assume, *a priori*, that a regulatory factor active in mouse ES cells necessarily exhibits the same function in their human counterparts.

We showed that RNAi can be used effectively to down regulate genes in a specific manner in hEC and hES cells, and also OCT4 expression is required to maintain the undifferentiated state of the human EC and ES cells. Further, we used RNAi to knockdown expression of *OCT4* gene in several hEC cell lines derived from human testicular germ cell tumours. These included 2102Ep, 833KE, 1156QE, 1777N and TERA1. 1777N, has been reported to be capable of significant somatic and extra-embryonic differentiation. In contrast, 2102Ep, 833KE, 1156QE and TERA1 no longer show significant somatic or extra-embryonic differentiation. Here we show that elimination of OCT4 protein expression results also in the differentiation of all these human EC cells, which is marked by changes in their surface antigens. RT-PCR results showed that these cells are differentiated towards trophectoderm and also *OCT4* RNAi resulted in attenuation of their cell growth. This opens up the possibility of targeting genes known to play a role in pluripotency or malignancy, using RNAi technologies, in cancer therapy.