DNA damage pathways in mouse pre-implantation embryos

A.A. Lim, H.-.S. Lee, M.L. Day, Department of Physiology and Institute for Biomedical Research, University of Sydney, Sydney, NSW, Australia

The survival of the pre-implantation embryo depends on the correct regulation of cellular proliferation. Investigations on how the cell cycle is regulated are therefore important. Previously we have shown that a K^+ channel whose activity is linked to the cell cycle is present in mouse early embryos[1]. Further studies showed that the fall in K^+ channel activity that normally occurs at the G1-S transition of the cell cycle was prevented in early embryos by DNA damage[2]. In order to determine which DNA damage pathway was responsible for the constitutive K^+ channel activity, an investigation into which DNA damage pathways operate in the pre-implantation embryo was performed.

Our preliminary studies using microarrays have shown that p38MAPK transcripts are elevated following treatment of mouse trophoblast stem (TS) cells with aphidicolin (APC), a DNA synthesis inhibitor. Therefore we investigated whether APC induced changes in p38MAPK protein and mRNA levels. Protein and RNA was extracted from mouse TS cells and 2 and 4-cell stage embryos[3] following treatment with 2μg/ml APC. Western blots were performed on total protein lysates and following immunoprecipitation with an antibody against phosphorylated (active) p38MAPK. After transfer, nitrocellulose membranes were probed with antibodies against p38αMAPK and p38βMAPK. For RT-PCR, isolated RNA was DNase-treated then reverse transcribed into cDNA. Expression of p38MAPK mRNA was then examined.

Preliminary results from this study reveal that protein and mRNA levels of p38MAPK are elevated following APC treatment of TS cells. Therefore a stress response pathway that acts via p38MAPK may function during early development.

1. Day, M. L., Pickering, S. J., Johnson, M. H. and Cook, D. I. (1993) Cell-cycle control of a large-conductance K^+ channel in mouse early embryos. Nature, 365: 560-562.
2. Day M. L., Johnson M. H. and Cook D. I. (1998) A cytoplasmic cell cycle controls the activity of a K^+ channel in pre-implantation mouse embryos. EMBO Journal, 17: 1952-1960.
3. Mice were killed by cervical dislocation prior to embryo isolation, in accordance with the NHMRC Guidelines on Ethics in Animal Experimentation.