Time-lapse study reveals bottlenecks in stem cell expansion

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A time-lapse study of human embryonic stems cells has identified bottlenecks restricting the formation of colonies, a discovery that could lead to improvement in their use in regenerative medicine.

Biologists at the University of Sheffield's Centre for Stem Cell Biology led by Professor Peter Andrews and engineers in the Complex Systems and Signal Processing Group led by Professor Daniel Coca studied human pluripotent stem cells, which are a potential source of cells for regenerative medicine because they have the ability to produce any cell type in the body.

However, using these stem cells in therapies is currently hampered by the fact they can acquire genetic changes during prolonged culture which are non-random and resemble mutations in cancer cells.

Researchers used time-lapse imaging of single human embryonic stem cells to identify aspects of their behaviour that restrict growth and would be targets for mutations that allow cells to grow more efficiently.

Dr Ivana Barbaric, from the University of Sheffield's Department of Biomedical Science, said: "We study pluripotent stem cells, which have huge potential for use in regenerative medicine due to their ability to become any cell in the human body. A pre-requisite for this is maintaining large numbers of undifferentiated cells in culture. However, there are several obstacles such as cells tend to die extensively during culturing and they can mutate spontaneously. Some of these genetic

mutations are known to provide stem cells with superior growth, allowing them to overtake the culture – a phenomenon termed culture adaptation, which mimics the behaviour of cancer cells.

"In order for pluripotent stem cells to be used safely in regenerative medicine we need to understand how suboptimal culture conditions, for example culturing cells at low split ratios, affect the cells and can lead to culture adaptation."

The team's research combined the use of time-lapse microscopy, single-cell tracking and mathematical modelling to characterise bottlenecks affecting the survival of normal human embryonic stem cells and compared them with adapted cells.

They identified three major bottlenecks affecting colony formation: survival after plating, failure to re-enter into cell cycle and continued cell death after division.

In the same culture condition, they found adapted cells performed better in all of these points leading to more colonies. Bottlenecks were also alleviated through cell to cell contact and pro-survival compounds.

Dr Veronica Biga, from the University's Automatic Control and Systems Engineering Department, said: "To extract information about cell death, mitosis and movement, we developed new methods for analysing images and measuring numerous parameters from time-lapse videos."

She added: "We plan to further develop the methods from this study into an image processing and analysis software solution to be used for monitoring cell behaviour in applications such as screening culture conditions, drug discovery, monitoring and minimising the occurrence of genetic abnormalities directly through time-lapse."

The work is published today (Thursday 12 June 2014) in the journal *Stem Cell Reports*.

Provided by University of Sheffield