A structured environment helps to regulate nuclear architecture in breast epithelial cells.

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A question that has been lingering within the nuclear architecture field for some time is how much influence does the way cells are cultured affect the behaviour of the nuclear architecture contained within those cells? There are a few studies that have sought to address this problem by direct comparison of nuclear compartments in the same cells grown on a flat substratum in 2 dimensions compared with cells grown in 3 dimensions within extracellular matricies; and indeed differences in the nuclear organisation are elicited[1,2]. One 3D cell culture system that works well is for breast epithelial cells which readily form 3D acini in extracellular matrix (ECM) that are structurally relevant for studying breast tissue and cancer in vitro[3]. Indeed, Maya-Mendoza et al., published in this volume of Cell Cycle[4], compare the nuclear architecture of breast epithelial primary and MCF10A cells grown in conventional 2-dimensional monolayer cultures with the same cells cultured in 3 dimensions in acini within laminin-rich extracellular matricies. To inform on changes within the cell nuclei between the culturing systems the authors counted the number of nucleoli within each nucleus under the different conditions, the idea being that the lower the number of nucleoli, the less complex was the nuclear architecture with respect to nucleoli. MCF10A cells grown in 2D displayed multiple nucleoli for quite some time in culture whereas the MCF10A cells grown in 3D had fewer nucleoli to start with and very quickly the majority of the cells contained only a single nucleolus. These data thus do indicate that the microenvironment within which cells are grown can affect their nuclear architecture. Significantly, 2D cultures grown on various ECM components or induced to differentiate had little impact on their nucleolar number. Furthermore, these data are in broad agreement with an earlier study that have grown MCF10A.B2 cells within ECM and shown that nucleolar number is very much reduced in cells in 3D conditions compared to 2D and that the reduction was not just due to cells leaving the cell cycle and becoming quiescent[5].

The Maya-Mendoza paper does however, describe how the 3D culturing of the breast epithelial cells brings about a “resting state” within the cells of the acini that is reversible but also displays hallmarks of classical irreversible replicative senescence such as senescence-associated-β-galactosidase staining. This state is correlated with a low number of nucleoli which increase in number when the senescence-like state is reversed by the explantation of the cells. The Maya-Mendoza study goes further to ask the question how is the cellular microenvironment able to stimulate structural alterations within the nucleus? The authors postulated that the mechanism may be mediated by β-integrins to transduce the information through to the cell nuclei. By blocking integrin activity with a specific antibody demonstrated that the simplification of nucleoli did not occur, even after a fortnight in culture.

Interestingly, when MCF7 breast adenocarcinoma cells were grown in the 3D acini cultures they did not respond to similar cues from the ECM and continued to contain multiple nucleoli and did not enter the resting state observed for the MCF10A cells. However, in 2D cultures when treated with aphidicolin to block DNA synthesis, MCF7 cells did respond by leaving the cell cycle but without simplifying their nucleolar number. Thus, it appears in breast cancer cells that any signalling mechanism influencing nuclear architecture from the cellular microenvironment is no longer

working in the same way. Losing the ability to control the nuclear contents and genome in cancer cells would have dire consequences in an in vivo situation.

This study certainly adds to the growing number of studies that suggest growing cells on 2D substratum is no longer quite as acceptable as it was and links 3D culturing with structural and organisational responses deep within the cell nuclei.

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