3 - 102 Differential Expression of miRNA between Monolayer and Three Dimensional Cells after Ionizing Radiation

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Many important advances in the fields of biology and medicine are acquired based on two dimensional (2D, monolayer) cell culture studies or on whole-animal model systems. However, when grown in traditional culture such as on flat 2D tissue culture substrates, cells do not recapitulate the structural organization and differ considerably in their morphology, cell-cell and cell-matrix interactions, and differentiation from those growing in more physiological 3D environments. As an another experimental continuum, animal models may not adequately reproduce features of, for example, human tumors, drug therapeutic responses, autoimmune diseases, and stem cell differentiation[1]. To overcome the above mentioned problems using 2D cell culture system and animal models, a novel technology has been developed to culture three dimensional tissues in vitro. Cells were plated in a certain scaffold, and a structure like the tissue in vivo will be formed in vitro. In vitro 3D tissue models provide a third approach that bridges the gap between traditional cell culture and animal models.

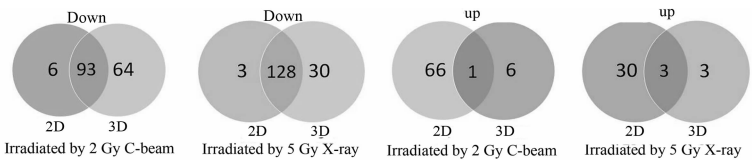

Fig. 1 MiRNA expressions were detected in 2D and 3D structure cells 30 minutes after exposed to C-beam and X-ray irradiation using miRNA chip assay. 5 Gy X-ray is equal to 2 Gy C-beam in relative biological effect dose. More miRNA regulated down in both 2D and 3D cells after irradiation.

A human lung cell 3D model has been used to assess the responses of cells to DNA damage induced by low- and high-LET ionizing radiation (IR) by our group. It was found that the 3D structures were less sensitive to IR than cells in 2D and DSBs were repaired with slower kinetics in 3D structures[2]. Another example of this phenomenon is enhanced tumor cell resistance to radiotherapy and chemotherapy in 3D cultured cells[3].

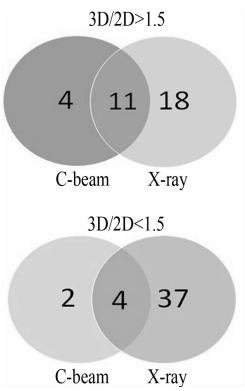

Fig. 2 Compare the expression of miRNA between 2D and 3D cells after irradiation.

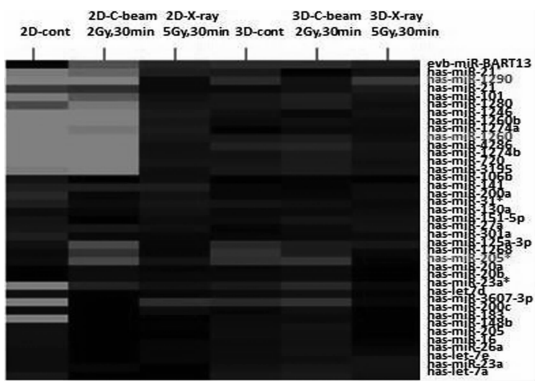

Fig. 3 Hotmap of miRNA expression in 2D and 3D structure cells.

What is the mechanism involved in this difference? Cultivation mode change may cause epigenetic regulation changes, which impact gene expression without genetic change between 2D and 3D cells although they have the same genetic background. MicroRNA (miRNA) are a single-stranded non coding RNAs of 19-25 nucleotides in length, generated from endogenous transcripts that contain a local hairpin structure. It also play a certain regulatory role in epigenetic regulation. Meanwhile, miRNA is closely related with

tumor in a variety of pathological processes by adjusting the proto-oncogene or tumor suppressor gene expression.

Our primary experimental results showed that more miRNA in 3D human lung epithelial cells (3KT) down regulated than in 2D 3KT cells after not only X-ray but also C-beam irradiation using the miRNA chip assay (Fig. 1). X-ray induced more significantly differential expression of miRNA when the relative expression value of miRNA in 3D cells were compared to 2D cells after irradiation (Fig. 2).

Further work will focus on the significantly differential expression of miRNA such as has-mir-1260, hsa-mir-1290, has-mir-205* as shown in Fig. 1. These defferential expression of miRNAs will be verified by real time-PCR (qRT-PCR) between 2D and 3D cells after irradiation. The biological functions of verified miRNA will be investigated through constructing high expression vector and inhibitors, etc.

References

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[3] S. Katja, E. Iris, B. Kerstin, et al., Cancer Res., 70, 10(2010)3925.