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T-3. Optimization of in vitro model for analysis of tumor cell migration dynamics

A. O. Kravchenko[1, 2], V. R. Kosach[1], K. A. Shkarina[1], I. V. Zaiets[1, 2], I. O. Tykhonkova[1], A. I. Khoruzhenko[1]

[1] Institute of Molecular Biology and Genetics, NAS of Ukraine 150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03143; [2] Educational and Scientific Center "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv 64/13, Volodymyrska Str., Kyiv, Ukraine, 01601
tykhonkova@gmail.com

Migration ability is an important feature of tumor cells. There are several approaches to analyze the dynamics of cancer cell migration *in vitro*. One of the most perspective and closer to the *in vivo* conditions is the model of initiation of the cell migration from 3D multicellular spheroids onto growth surface. Aim. Optimization of the model for adequate quantitative characteristics of the tumor cell locomotion during several days. Methods. 2D and 3D MCF-7 cell culture, immunofluorescence analysis, and image analysis using computer software Fiji. Results. Unification of spheroid size allowed avoiding a significant data deviation. The obtained spheroids spread completely for 3 days. The highest migration ratio was observed at the 2nd day. The proliferation level at each of 3-day experiment was the same and did not exceed 3 %. The validity of the model was tested after migration inhibition by rapamycin (mTOR signaling inhibitor). Additionally, this model was successfully applied to immunofluorescence analysis, namely investigation of p85S6K1 subcellular localization in moving MCF-7 cells. Conclusions. Double filtration of multicellular spheroids allowed unification of their size, which promotes an adequate interpretation of the migration assay. This model enabled the study of tumor cells migration dynamics and can be further used for the development of anticancer drug.

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U-1. The involvement of DNA damage response pathway in nuclear reorganization during netotic initiation

Burhan Uyanik[1], Bogdan B. Grigorash[1,2], B. Uyanik[1] , Oleg N. Demidov[1,2]

[1] INSERM 1231, University of Burgundy Franche-Comte, Dijon, France; [2] Institute of Cytology RAS, Saint-Petersburg, Russia
uyanik.burhan@hotmail.fr

The netotic type of cell death plays an important role in innate immunity and host defense. It is characterized by epigenetic changes in chromatin modifications, nuclear envelope disassembly, chromatin expulsion and formation of extracellular chromatin nets loaded with anti-bactericidal granules containing aggressive proteinase and pro-oxidant enzymes. Recently, it was shown by several groups that this process has a negative impact on tumor progression. Methods: To study

an involvement of DNA damage response pathway in nuclear reorganization during netotic initiation we selected an HL-60 based model. HL-60 is an acute myeloid leukemia cell line that preserved the capability of differentiation into granulocyte cell lineages. At first step, we performed differentiation of HL-60 cells into neutrophils with DMSO or ATRA. On the fifth day of differentiation, we have applied phorbol myristate to induce netotic cell death. Alternatively, we have treated granulocyte fraction isolated from bone marrow and blood of wild-type mice or from human donor blood with phorbol myristate. To study involvement of DNA damage response pathway we used an inhibitor of Wip1 phosphatase. Wip1 is a DNA damage induced nuclear phosphatase and a major negative switch of several DNA damage response proteins such as ATM, Chk1, Chk2, p53 and others. Results: Recently, several studies have suggested that nuclear structure of neutrophils plays an important role in their survival, their ability to migrate and control the release of NETs. First, we observed that Wip1 phosphatase expressed at high levels in the nucleus of differentiated HL-60 myeloid cells and in neutrophils. We observed that the inhibition of nuclear phosphatase Wip1 activity could extend mouse and human neutrophils and differentiated HL-60 cells survival through dephosphorylation and inhibition of its target proteins. Additionally, neutrophils treated with Wip1 inhibitors tends to show more segmented nuclei which have been correlated before with better anti-tumoral immune functions. Conclusions: In this context, we discovered that the inhibition of nuclear phosphatase Wip1 activity promotes neutrophil migration and control critical functions associated with nuclear reorganization during netotic type of cell death. The targeting of nuclear phosphatase Wip1 in neutrophils could be a new therapeutic strategy in the treatment of several diseases that trigger the inflammatory response and netotic type of death.

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V-1. Organization of zebra finch oocyte nucleus

V. Volodkina[1], A. Saifitdinova[1,2], M. Kulak, E. Koshel[3], S. Galkina[1], E. Gaginskaya[1]

[1] Saint Petersburg State University, Saint Petersburg, Russia; [2] Herzen State Pedagogical University of Russia, Saint Petersburg, Russia; [3] ITMO University, Saint Petersburg, Russia
leravolo94@gmail.com

Transcriptionally active lampbrush chromosomes and numerous of different intranuclear structures make nuclei of birds' growing oocytes a working model for investigation of nuclear structure and function. Studies of nucleus content were already made for chicken (Gallus gallus), quail (Coturnix japonica), chaffinch (Fringilla coelebs) and rock pigeon (Columbia livia). Here, we examine the organization of zebra finch (Taeniopygia guttata) oocyte nucleus and look at intranuclear structures. Zebra finch is a singing bird of Estrildidae family that belongs to Passeriformes. This bird is a well-known model object of neurobiology. To implement our idea we combine the methods of nuclei isolation, confocal microscopy and immunocytochemistry. We found out that there are a lot of (~ 40) small bodies (less than 0,5 μm) on the