VIDEOMICROPY OF LIVING CELLS IN VITRO

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Time-lapse microscopy of living cells (TLMLC) is intended for photographing cells and subcellular structures at discrete time intervals. This experimental approach allows to study the dynamics of different cellular events and insures an efficient data-mining in comparison with experiments using fixed ("dead") cytological specimens. Time periods of TLMLC experiments and their technical details are defined by the process under investigation. For monitoring intracellular events relatively short times (minutes – hours) are used, and fluorescent molecular analysis may be applied. However, long periods of uninterrupted cell monitoring are necessary for analyzing such processes as formation of cell clones and changes of locomotor activity, shape and size of cells during ageing. In long-term experiments application of fluorescent imaging is problematic due to harmful effects on cells of dyes and excitation light.

In our study videomicroscopy of living cells was used for understanding the cause of differences of lifespans and cancer transformation in man and mouse [5]. The man and mouse are mammalian species with great differences in lifespan and the rate of development of cancer diseases. The detailed comparison of human and mouse somatic cells in the context of ageing and cancer transformation can be achieved by study of individual cells and cell clones with the computerized videomicroscopy of living cell cultures. The analysis of videofilms revealed the prominent difference between human and mouse fibroblasts. In mouse big cells (up to 8000 mkm^2) entered mitotic division as frequently as smaller cells. In contrast, in human cell cultures only small cells (whose area did not exceed 2300 mkm^2) divided. The cell size increase is typical for cellular ageing, and genomic damage accumulates in aged cells. In particular, at least some big cells may be polyploids which can produce aneuploid progeny. In mouse we found out the process of a fast cell rounding without a following mitotic division but with a subsequent spreading and cell size increase. This process may be connected with replication of DNA without division, i.e. with polyploidization. It is possible that in man there is a relatively more strict system of blocking of mitotic divisions in abnormal cells, including big polyploid and aneuploid cells. This blocking system retards the accumulation of dysfunctional (aged) as well as transformed cells. It can be one of the reasons of the lower rate of cellular and organismal ageing and later development of cancer in man in comparison with rodents.

In another study we employed real-time imaging of living cells for investigating the mechanisms of self-renewal of immortal cell populations. The causes of the indefinite propagation of immortalized cell populations remain insufficiently understood, that hinders the research of such fundamental processes as ageing and cancer. In our study the interrelations between clonal proliferation and abnormalities of mitotic divisions in the immortalized cell line established from the mouse embryo were investigated. 3 mitoses with three daughter cells and 7 asymmetric mitoses which generated two daughter cells of conspicuously different sizes were registered among 71 mitotic divisions in the individual cell genealogy. Abnormal mitotic divisions either did not slow the proliferation in cell clones compared with progenies of cells that divided by means of normal mitoses or were followed by the acceleration of divisions in consecutive cell generations. These data suggest that abnormal mitotic divisions may contribute to the maintenance of the immortalized state of cell populations by means of generating chromosomal instability.

We also used computer videomicroscopy of live cells for establishing the cell line from human hair follicles and selection of culture conditions that induce a symmetrical neural differentiation of these cells.

In cooperation with the Minsk-based plant "Planar" we created a new computer videcomplex "Tsitomir" which performs a simultaneous videorecording of many areas of cell culture. The videocomplex can find broad application in biotechnological research.