THE ROLE OF INTERFERON AS A MODIFIER OF EPITHELIAL-MESENCHYMAL TRANSITION IN TUMOR CELLS

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The epithelial to mesenchymal transition (EMT) is the process characterized by the loss of epithelial characteristics and the acquisition of mesenchymal attributes by epithelial cells. EMT provides the progression of cancer cells to the metastatic phenotype and contributes to epithelial tumor cells to migrate, invade into the surrounding stroma and spread to secondary organs [1]. Classification of EMT into three different subtypes EMTs are encountered in three distinct biological settings that lead to different functional consequences:

- EMT that occurs during embryogenesis and development of organs;
- EMT associated with regeneration of tissue and organ fibrosis;
- EMT associated with tumor progression and metastasis.

A proposal to classify EMTs into three different biological subtypes based on the biological context in which they occur was discussed at a 2007 meeting on EMT in Poland and a subsequent meeting in March 2008 at Cold Spring Harbor Laboratories. We have paid special attention to the third type of EMT, which includes some certain patterns such as:

- enhanced migratory capacity, invasiveness;
- elevated resistance to apoptosis;
- greatly increased production of ECM components.

These changes include activation of transcription factors, expression of specific cell-surface and cytoskeletal proteins, production of ECM-degrading enzymes, and modulation of the expression of specific microRNAs [2, 3].

It is known that epithelial and mesenchymal cells have been identified on the basis of their morphology: typical epithelium is a sheet of cells with individual epithelial cells abutting each other in a uniform block. Regularly spaced cell-cell junctions and adhesions between neighboring cells hold them tightly together and inhibit the moment of individual cells away from the epithelial monolayer. On the other hand, mesenchymal cells usually exhibit neither regimented structure nor tight intracellular adhesion and these cells form structures with irregular shape. Adhesion between mesenchymal cells is not so strong than in epithelium and mesenchymal migration is mechanistically different from epithelial one: mesenchymal cells move individually more dynamically than epithelial cells, which move as a sheet in block [4]. Today there are many markers that characterize these types of cells at the molecular level. The transformation of epithelial cells to mesenchymal requires changes in morphology, cell adhesion and the ability to migrate.

The understanding of the processes of EMT and mesenchymal-epithelial transition (MET) to tumor progression and monitoring of these processes in clinical practice is of great importance, as well as the search for modifiers of these processes, as it will provide new targets for treatment and help to change routine schemes of therapy [5, 6].

According to our opinion, such a modifier can be interferon. More than a half a century ago, interferons (IFN) were identified as antiviral cytokines. Since that discovery, IFN have been in the forefront of basic and clinical cytokine research. The pleiotropic nature of these cytokines continues to engage a large number of investigators to define their actions further. Modern studies have demonstrated that IFN is a polypotent cytokine that is able to suppress proliferation of malignant cells, and sometimes is causing their direct lysis; it causes suppression of angiogenesis in tumor tissue and stimulates specific immune response and nonspecific antitumor resistance of an organism; IFN possesses antimutagenic activity, promotes apoptosis in tumor cells induced by different agents; it suppresses motility of tumor cells and expression of oncogenes; IFN influences the mechanisms of tumor cell differentiation [7–9]. Also, it has been shown that IFN modifies interactions between the cells and with different biologic substances: antibodies, lymphokines, cytokines, hormones, growth factors, etc.

Earlier using the murine cells transformed with viral oncogenes it has been shown that IFN suppresses malignancy of transformed cells and this effect of IFN may have been related to the changes in the methylation of viral and/or cellular genes. The ability of IFN to reverse malignant phenotype of tumor cells is manifested in a long-term action of this cytokine on human tumor cells in vitro [10].

In our study we have used the experimental model of prolonged exposition of tumor cells to IFN because

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Abbreviations: ECM — extracellular matrix; EMT — epithelial to mesenchymal transition; IFN — interferon; MET — mesenchymal to epithelial transition.
we have taken into account the clinical schedules of long-term courses of interferon therapy; from other hand, the data on IFN influence on numerous patterns of the cells has been reported for short-term exposition of the cells to IFN, after which the modified properties of cells were renewed.

The ability of IFN to induce suppression of tumor cells malignancy is in some way related to its well known ability to stimulate tumor cell differentiation. Recently it was also shown that IFN inhibits the processes of fibrosis in vivo [11]. The above brief description of the IFN activities directly points on its possible involvement in control of the process of EMT and induction of MET.

Such assumptions we’ve proposed because IFN also has a permanent effect on the cells in organism where it is an endogenous factor, which can be activated in different situations for control of homeostasis.

In our work we have used non small cell lung cancer cells of A-549 line, HeLa — cervical carcinoma, K-562 — chronic myelogenous leukemia obtained from the Bank of Cell Lines from Human and Animal Tissues (Kyiv, Ukraine), which were cultured in complete RPMI 1640 culture medium (Sigma, USA) supplemented with 4 mmole/L L-glutamine, 10% fetal calf serum (Sigma, USA), and 10% of 4°C serum. The cells were cultivated in humidified 5% CO2 atmosphere at 37°C, the medium was replaced replaced each two days, and the cells were passaged each four days. To study the long-term effect of IFN, the cells were cultured for more than 2 month (A-549 — for 1 year) in the presence of recombinant IFN-alpha-2b (BioPharma, Ukraine) at increasing concentrations (from 100 to 10000 U/ml).

The expression levels of E-cadherin, N-cadherin, vimentin and actin (smooth-muscle actin) proteins were analyzed using immunocytochemical approach with the use of monoclonal antibodies (Dako North America, BioLegend, Diagnostic BioSystems).

Our previous study of growth kinetics of A-549 and K-562 cells and recent studies of HeLa cells that were cultivated with or without IFN in vitro has shown significant alterations of growth patterns of the cells exposed to IFN [12]. In particular, it has been revealed that along with prolongation of cell exposition period and elevation of IFN concentration, the period of cell duplication becomes longer, cells became larger and some of them demonstrated enhanced manifestation of epithelial morphology. We also have shown that prolonged cultivation of A-549 cells with IFN leads to dramatic decrease of the number and size of colonies of A549 cells in semisoft medium; IFN-modified tumor cells lost an ability to grow in serum-free culture medium; also, we have revealed a decreased expression of EGF-R and VEGF. So, all these acquired abilities of IFN-modified cells indicate a significant reduction of their tumorigenicity.

We showed that the long-term exposition of A-549 adenocarcinoma cells to IFN-alpha leads to reliable decrease in the number of cells expressing vimentin and leads to strong increase in the number of cells, which express E-cadherin (Table). At the same time we revealed that the long-term exposition of A-549 cells to IFN leads to decreased number of N-cadherin-positive cells. It is known that E-cadherin is responsible for maintaining interactions of epithelial cells and is frequently downregulated during tumor progression, while N-cadherin that is normally found in fibroblasts and neural cells, can be upregulated during tumor progression and can increase the invasiveness of tumor cells [13]. We have registered increase of E-cadherin expression and decrease of N-cadherin and vimentin expression after prolonged treatment of HeLa and K-562 cells with IFN (Fig. 1, 2, see Table). From these data we can conclude that IFN can be involved in the control of EMT/MET programs of tumor cell transdifferentiation because all studied proteins are the markers of this process.

So, we have shown that long-term treatment of human cancer cells by IFN-alpha leads to alterations in expression of epithelial and mesenchymal markers, which indicate inhibition of EMT program and activation of MET program. This phenomenon correlates with inhibition of malignant phenotype of cancer cells. It opens a novel direction of investigation of the IFN — one of the most important regulator of homeostasis in systemic level and genetic stability on cellular level, especially in a situation where almost nothing is known about this cytokine as a modifier of EMT.

Our hypothesis requires further evidence. In general, it is a good explanation of many experimental data and clinical effects of IFN, which are attributed to the other properties of this cytokine. Search in this way helps us find new and important target for the suppression of aggressive behavior of cancer cells.

**REFERENCES**


**Table.** Change of some EMT protein marker expression in the IFN-treated tumor cells

<table>
<thead>
<tr>
<th>Nº</th>
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<th>A-549</th>
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<th>K562</th>
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<td></td>
<td></td>
<td>control, %</td>
<td>IFN-modified, %</td>
<td>control, %</td>
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<td>Alpha-smooth muscle actin</td>
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<td>87.3±2.2</td>
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Fig. 1. Immunocytochemical analysis of expression of E-cadherin in HeLa (a, b) and K-562 (c, d) cells: control (a, c), IFN-modified cells (b, d) (x1000)

Fig. 2. Immunocytochemical analysis of expression of N-cadherin in HeLa (a, b) and vimentin in K-562 (c, d) cells: control (a, c), IFN-modified cells (b, d) (x1000)


