

Differentiation Therapy by Extracts of Embryo Cells: A Possibility for Cancer Treatment

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Abstract

Various chemical and biochemical differentiation compounds have been shown to revert to normal state several kinds of cancer cells. As embryonic and cancer cells present similarities of behavior and phenotype and as regulatory factors in young embryos have been found, a treatment of cancer cells by embryo cell extracts could be possible. These factors were prepared from rat and mouse embryonic cell nuclei and partially purified by high performance liquid chromatography. They inhibited up to 90% DNA synthesis of LFC12A, an established cell line from a hepatocarcinoma and of Raji, a B cell line at the stage blast 1 from a Burkitt lymphoma. They prolonged the survival time of rats injected with LFC12A and increased the percentage of survivors at one month. In the same way, the inhibitory and apoptotic effects of Zebra fish proteins on human colon cancer cells and hepatocellular carcinoma have been reported by Italian researchers. As these factors were not cytotoxic and were active at low concentration, they could be used repeatedly on the remaining cancer cells, after surgery, radiotherapy or chemotherapy.

Keywords: Cancer cells; Differentiation therapy; Chemical and biological compounds; Embryonic nuclear proteins

Introduction

Surgery, chemotherapy, and radiotherapy, (at total 88.5% of the studies) [1], are the main methods used for the treatment of cancer, owing to a mean of 64% of the cancer patients survive at least 5 years after their diagnosis [2], with a huge variation in survival between cancer types. Their aim is to remove or to kill as many cancer cells as possible, while preserving the normal tissues, their flaw is to leave inevitably some cancer cells or to select treatment-resistant cells. The percentages of articles about surgery, radiotherapy and immunotherapy are slightly decreasing, the one about chemotherapy is constant, while those about gene therapy, by introducing tumour suppressor genes (6 % of the studies), and differentiation therapy, by chemical or biological compounds (2 % of the studies, which is the subject of this review) are increasing.

Cancer Cell Differentiation by Chemical and Biological Compounds

Erythroblast leukemic cells in culture have been differentiated for the first time by dimethylsulfoxide (DMSO) [3]. Many other biological compounds, such as all-trans (ATRA) and 9-cis retinoic acids [4-11], interferons [5], granulocyte colony stimulating factor (G-CSF) [5,6], prostaglandin E2 [9], transforming growth factor β (TGF β) [12], differentiation inducers such as MGI 2, have been tested on myeloid leukemic cells [13-17]. They have efficiency, alone or in combination [9,10,12], sometimes very high: a 90-95 % complete remission, 5-year survival time probability of 0.71 in the case of acute promyelocytic leukemia treated by ATRA [11]. The survival time of mice injected with cancer cells is prolonged by treatment by these products. However, this method is limited to particular types of cancer, the results depend on the clones of the malignant cells used. Differentiation therapy, out of the treatment of leukemia by retinoic acids, was progressively abandoned and no more mentioned during a decade.

Embryonic Characteristics of Cancer Cells

Cancer cells present similarities of phenotype and behaviour with their embryonic precursors and most of cellular oncogenes are normally expressed during ontogeny: the concentration of *c ras* is maximum in the mouse embryo at the 15th day, that of *c abl* around the 10th day, and of *c fos*, before the 9th day [18]. The human oncogene *c amv* is present in all immature myeloid and lymphoid T cells, but not in mature B and T cells and found only in small quantities in leukemic cells differentiated by DMSO or retinoic acid [19]. The carcino embryonic antigens expressed in the tumours of the human digestive system are normally present in the corresponding fetal tissues [20]. By another way, certain genes active in the embryo are oncogenic: when dispersed embryo cells are grafted on adult animals, or set in tissue culture, they form special tumours called teratocarcinomas [21]. In this case, tumour formation does not need any mutation, carcinogenesis is induced by the mere disorganisation of the embryo.

Regulatory Factors in Embryonic Cells

The embryo contains differentiation factors that regulate cellular gene expression and enable the normal development of the different organs. This ability of regulation can be applied to certain types of cancer cells: a blastocyst regulates one injected teratocarcinoma cell consistently, which contributes thereafter to the normal tissues of the adult [22,23]. However, the results are less decisive when several cells are injected together or when B16 melanoma cells are used [24].

Similarly, when injected into 10 day old mouse embryos, myeloid leukemia cells lose their malignant character, and the apparently healthy adult mice have granulocytes containing a marker derived from the cancer cells [25]. This regulating capacity of mouse embryos decreases with age: towards myeloid leukemic cells, it disappears after the 11th day [25].

Thus, it seems that differentiation factors, probably proteins, are present in the young embryo and are able to revert certain types of cancer cells into normal, non pathogenic, differentiated ones. They are in general absent or ineffective in adult animals, since ascite tumours or teratocarcinoma develop in adult mice. In the latter case, some cells differentiate (teratoma), while the other ones divide rapidly (carcinoma), likely by depletion of regulatory factors from the embryo following the destruction of its structure and by lack of differentiation factors from the adult receiver.

The factors of differentiation working by binding on DNA must be mainly localized in the nuclei of the cells of the embryo [26]. Moreover, the separation of the nuclei has the advantage of eliminating the cytoplasmic proteins.

Extracts of Rat and Mouse Embryo Cell Nuclei

This hypothesis has been tested with rat and mouse embryo nuclei [27,28], prepared by a modification of the method of Widnell and Tata [29] and the activity of their extracts have been tested on LFC₁A, an established cell line derived from a hepato carcinoma induced in the Commentry rat by 4 dimethylamino-azobenzene (Villejuif, France) and on Raji, a B cell line at the stage blast 1 originating from a Burkitt lymphoma which expresses EBV early viral antigens. [30,31].

To evaluate the biological effect of the different fractions studied, the variation of the DNA synthesis, measured by the incorporation of ³H thymidine, was used: the DNA synthesis was inhibited, up to 90%, compared to control.

In all experiments, the number of hepatoma cells after 48 hours incubation with the extracts is superior to the initial number of cells, showing that the material presents no cytotoxicity. The comparison of the number of cells and the incorporation of radioactivity shows that there is inhibition of DNA synthesis per cell, then growth inhibition and modification of the metabolism of the cells.

In the in vivo experiments with young rats injected with 10⁶ cells of LFC₁A, the median survival time is increased by 17% when the cells have been incubated with the extracts (5 experiments, with a total number of 73 animals). This prolongation of survival is relatively reduced, but significant at the 1.5% level, with the Mann and Whitney test. The percentage of survivors at one month is multiplied by 3 after incubation with extracts.

The extracts presented in HPLC on TSK DEAE anion exchange column between 15 to 20 partially or completely resolved peaks of protein absorption spectrum (OD 280nm/OD260nm between 1.2 and 1.7). and a major peak, containing mainly DNA (maximum OD at 260 nm), eluted at high ionic strength. The latter contains in addition proteins, visible as 6-8 bands by polyacrylamide gel electrophoresis, of molecular weights comprised between 14 and 20 kD. These proteins have high affinity for DNA, since they elute with it by chromatography on anion exchange column, at high ionic strength (0.8 M NaCl in our condition). They are in minute amounts compared to DNA, the absorption ratio OD 280nm/OD 260 nm being 0.5. They are supposed to be constituted of histones and other DNA binding proteins, such as transcription factors, but further identification has not been pursued. Neither purified rat or mouse DNA, nor sonicated DNA were active on radioactive incorporation and DNase treatment of the extracts does not suppress the activity. These data eliminate DNA as a cause of inhibition of the ³H thymidine incorporation.

Some particular points must be emphasized

The corresponding oncogenes of different species (human, cat, rat, chicken, insects) present a great similarity [32]. One can conceive that the transcription factors have been conserved too during evolution and that it would be possible to regulate cancer cells of one species with differentiation factors from another one. Actually, we have observed that extracts of rat embryo cell nuclei were active on rat hepatoma and on human lymphoma cell line.

Several oncogenes are active in human tumor cells. Moreover, a cell becomes cancerous after multiple mutations and modifications of the expression of its genes and is always evolving during the course of malignancy. A mixture of factors coming from different immature tissues may have a stronger action on the expression of different oncogenes than the sum of the effects of the separated factors.

Except perhaps in the case of organ regeneration [33], normal differentiated cells do not produce (or not enough) regulatory factors able to revert cancer cells: injected ascite tumours, as spontaneous tumours, can generally develop in adults.

Conversely, are the extracts of embryo cell nuclei active towards the normal differentiated and stem or germ cells of the organism? This point ought to be the subject of further study and could eventually be solved by chromatographic separation.

Biological Activities of Zebrafish Embryo Extracts

The same approach has been followed by Italian researchers, who have obtained interesting results. Zebrafish embryo proteins induce inhibition of human colon cancer cells by enhancement of apoptosis in a dose dependent manner [34]. Cell cycle analysis in treated cells revealed a marked accumulation in the G(2)/M phase preceding induction of apoptosis and increase of caspase 3 and 8 activities.

Stem cell differentiation stage factors from Zebrafish embryos, during the stage in which totipotent stem cells are into pluripotent stem cells, have been shown to inhibit proliferation and induce apoptosis in colon tumours [35].

Out of 38 patients with advanced hepatocellular carcinoma, treated with these stem cell differentiation stage factors from Zebrafish embryos, 5 of them presented a sustained complete response, and no side effects occurred [36].

Current and future perspectives of this novel technology are discussed and a new hallmark of cancer is suggested: the loss of differentiation of cancer cells [37,38].

Conclusion

These data show that extracts from mouse and rat or Zebrafish embryo cells contain factors that inhibit the proliferation of cancer cells, prolong the survival time of rats injected with them, and enhance the apoptosis rate. It seems that they can be obtained from different species and purified by the general chromatographic methods. They are not cytotoxic since the number of cells incubated with them does not decrease, and not immunogenic, as they are in very low concentration. Consequently, this treatment could be repeated several times, in order to revert the remaining cancer cells, in the cases of treatments by surgery, radio or chemotherapies.

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