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Embryonic Differentiation Factors with Anticancer Properties: Preliminary Clinical Results in the Therapy for Advanced Tumors

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Experiments on animals have demonstrated that regulatory factors can stop or delay tumor growth. These regulators are present in the embryos of ovipara and in the pregnant uteri of mammals during differentiative processes and can regulate the anti-oncogene p53 and pRb in different tumor cells in vitro. These regulators are probably specific for each kind of tumor. A redundancy of these regulators was used in human therapy in an attempt to stop or delay the progression of the disease. The therapy consisted in the sublingual administration of low doses (from 9 micrograms/day to 12 micrograms/day of total proteins) of Zebrafish embryonic extracts at the stages of middle-blastula-gastrula, 5 somites, 20 somites in a glyceroalcoholic solution. The cases treated were seriously ill patients on whom oncologists had stopped traditional efficacious cancer therapies and patients with significant metastasis but still being treated with chemotherapy, radiotherapy or thermotherapy. Patients at the initial stages of disease were not included in this trial. In 3 years we treated 200 patients. Results: 1) 80% of the cases showed an improvement of the performance status, 2) the survival curves demonstrated a stabilization of the disease in a certain number of the final-stage cases 3) 8% of the cases demonstrated a regression of the tumoral masses. This clinical trial represents an open study and as such final conclusions cannot be drawn, except for the non-toxicity of the therapy. In fact, no adverse effects were observed in all the patients treated. A case-control study is now underway.

Introduction

It has been reported in literature (1,2,3,4,5,6) and demonstrated in vitro and in vivo (7,8,9, 10) that factors present during embryonic organogenesis can reduce or suppress tumor growth. This is probably because the substances taken from the embryo during cell differentiation can regulate important genes of the cell cycle. In fact, it was possible to activate the p53 onco-suppressor gene after treatment with embryonic extracts on different tumor cell lines in vitro (11). In addition to this

transcriptional regulation, a post-translational regulation of pRb on the human kidney adenocarcinoma cell line has been reported in another article in this issue. The evidence that embryonic factors of cell differentiation can be used as a" physiological gene therapy" of cancer constituted the objective basis to prepare a therapy to test in human cancer for compassionate use. As a result, different products containing specific embryonic differentiation factors, were prepared.

The aim of this paper is to illustrate the therapy and the preliminary results in humans.

Description of the therapy-Patients and methods

The products used for human therapy are Zebrafish embryo extracts in glyceroalcoholic solution.

Each solution has extracts of the embryos respectively at the stages of middle blastula-gastrula, 5 somites, 20 somites.

The Zebrafish embryos are washed in distilled water and placed in a solution of pure glycerin and 30 % ethylic alcohol. The proportion is 85 % and 15 % respectively.

The embryos are sonicated with 2 cycles of 10 seconds each and further treated with a turboemulsifier for 3 minutes.

These solutions are diluted with ethylic alcohol 30 % at the ratio 1 to 10: the final concentrations of total proteins must be about 3 micrograms/milliliter.

The therapy consists of the sublingual administration of 9-12 micrograms / day of total proteins obtained from the stages of middle-blastula-gastrula, 5 somites, 20 somites : the patients were usually given the substances from the middle-blastula-gastrula stage (66 %) and the substances from the other stages (34 %, ie17 % from the 5 somites stage and 17 % from the 20 somites stage).

The cases treated were :

1. seriously ill patients on whom oncologists had stopped traditional efficacious cancer therapies;
2. patients with significant metastasis but still under chemotherapy, radiotherapy or thermotherapy treatment.

Patients at the initial stages of disease and well controlled by traditional therapy were not included in this clinical trial.

In 3 years we treated 200 patients.

The following was recorded for each patient:

a) the performance status using the E.C.O.G. (Eastern Cooperative Oncology Group) system of evaluation.

b) the survival time from the beginning of the therapy. The Kaplan-Meier method was used to describe the survival curves.

c) The dimensions of masses of primary tumors and their metastases.

Results

The cases treated are shown in Table 1.

Tab. 1 - Cases treated with embryonic extracts.

60 breast cancer	8 pancreas cancer
23 lung cancer	6 myeloma
18 colon cancer	6 bladder cancer
15 hepatocarcinoma	4 ovary cancer
15 stomach cancer	4 leukemia (1 myeloid acute,
11 prostate cancer	3 myeloid chronic)
9 lymphomas (8 Hodgkin; 1 n.H.)	1 larynx cancer
9 glioblastoma	1 melanoma
9 kidney cancer	1osteosarcoma

60 of these cases were under thermotherapy treatment. 19 cases were under chemo-radiotherapy treatment.

80 % of the treated patients improved their performance status (generally from the 4 or 3 status of E.C.O.G. scale to the 2 or 1 status). In addition, the patients reported a reduction of pain and a decreased intake of analgesic drugs.

The masses of primary tumors or of their metastases decreased in the following cases: breast cancer (4 cases), lymphoma non Hodgkin (1 case), lymphoma of Hodgkin (1 case), cancer of the colon (2 cases), stomach (1 case), lung (1 case), kidney (1 case), bladder (2 cases), prostate (1 case under goserelin therapy), larynx (1 case), osteosarcoma (1 case).

The survival curves are reported only for the most numerous subgroups of cancers in the whole group.

Discussion

This clinical trial is an open study that is usually made before a case -control study. Final conclusions cannot be drawn without the control group, except for the non-toxicity of the therapy. In fact, no adverse effects were observed in all the patients treated.

Other observations can be made at the end of the three-year period. It can also be stated that the therapy has some positive effects on performance status. In fact, 80 % of the patients improved their performance status and reported less pain.

These consequences cannot be ascribed to the placebo effect only, because their incidence is higher than the placebo effect.

Conclusions about the reduction of tumoral masses are more difficult to make. In fact, the patients treated were at a very advanced stage of the disease. 60 of these patients were under thermotherapy and 19 under chemo-radiotherapy, the other 121 patients had just finished chemotherapy or radiotherapy. The effects on the dimensions of tumoral masses could be ascribed to chemotherapy or radiotherapy. However, a very advanced kidney adenocarcinoma, with significant pulmonary, bone and surrenalic metastases demonstrated a complete regression of tumoral masses, without chemo-radiotherapy. The complete remission of the disease is still present after four years from the beginning of the therapy.

The survival curves demonstrated a high percentage survival rate after several months for each kind of tumor. These percentage survival rates are high, if we consider that the diseases were very advanced and that the life expectancy in these cases was generally no more than 6 months. Therefore this therapy seems to have a positive effect on the survival of the patients.

However, in order to clarify these aspects of the problem we are now conducting a case-control study.

This therapy can be considered complementary to the other efficacious cancer therapies.

We are also studying how to improve its efficiency: each kind of tumor probably requires a specific regulation network.

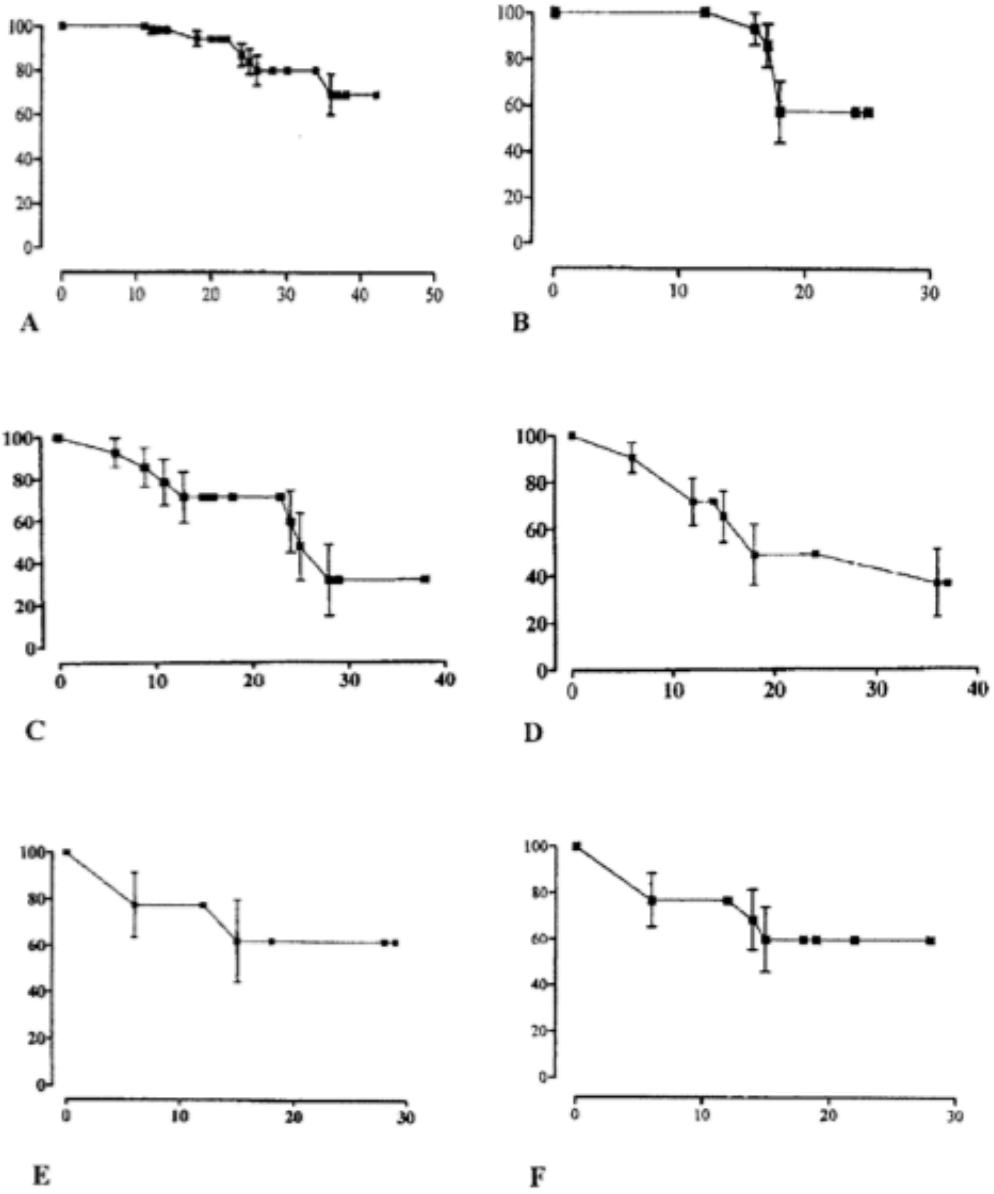

Figure: Percentage survival curves of patients with A) breast carcinoma; b) colon carcinoma; C) hepatocarcinoma; D) lung carcinoma, E) prostate adenocarcinoma; F) stomach carcinoma. Abscissa axis: months of treatment. Ordinate axis: percentage survival rate.

Fig. 1A shows the survival curve for 60 patients with breast cancer. After 40 months from the beginning of the treatment the percentage survival rate is about 80 %.

Fig. 1B shows the survival curve for colon cancer. The percentage survival rate is about 60 % after 24 months of treatment.

Fig. 1C shows the survival curve for hepatocarcinoma. The percentage survival rate is about 35 % after 40 months of treatment.

Fig.1D shows the survival curve for lung cancer. The percentage survival rate is about 40 % after 36 months of treatment.

Fig. 1E shows the survival curve for prostate cancer. The percentage survival rate is about 60 % after 30 months of treatment.

Fig. 1F shows the survival curve for stomach cancer. The percentage survival rate is about 60 % after 30 months of treatment.

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