Stem Cell Differentiation Factors as support in chemotherapy: Integrative treatments in oncology

Introduction

Edited by prof. Michele Carruba, Chairman of the Scientific Committee, Director of the Department of Pharmacology of University of Milano.

Discovering having a tumor is a dramatic event that makes the patient fragile and disoriented.

Physicians can not rule out the application of classical protocols and then each one follows his patients with the solercy, attention and human charge he is capable of.

Sometimes some patients feel alone, do not feel the benefit of chemotherapy and all the standard treatments and goes so depressed. They look at the internet, searching for other ways, and find alternative / integrative solutions like an appropriate diet, a new lifestyle or another doctor who listens to you more, supports you, and recommends supplementary products that were not previously proposed.

But the Internet is also full of inaccurate news and the risk for the patient to rely on unnecessary or worse harmful solutions is very high. In this context, where news goes on the web today much faster and without control, it is increasingly common that information from the world of research comes first to patients who are physicians, perhaps misbehaved: the risk of unnecessary theories and therapies It is so much higher.

For this reason, I felt the need to set up a scientific committee with the aim of studying and evaluating innovative researches in the epigenetic reprogramming of tumor cells with stem cell differentiation factors so as to avoid spurious or partial news dissemination and help University and clinical colleagues to best frame the research topic and its prospects for the future. We have carefully analyzed all the available scientific literature and identified those aspects of value that need to be further studied in the interest of both research and, above all, patient health.
I hope this short text can be a valid support in the orientation of all doctors and researchers interested in deepening this search path.

Stem Cell Differentiation Factors as integrative treatments in Oncology: their role in increasing the efficacy of chemotherapy and in reducing its adverse effects.

Parallel to the sequencing of the genetic code, another area of research, that of epigenetics, emerged. There are therefore two types of code, the genetic and the epigenetic code that is above the genetic code and regulate its functioning. In the field of epigenetic approaches, and in particular in the field of cancer, the research on the reprogramming of tumor stem cells with embryo differentiation factors has been consolidating for several years. In fact, it has been shown that the differentiation factors of stem cells extracted from Zebrafish egg, which has over 90% of common proteins with human ones, are able to normalize the cell cycle of cancerous cells. These are the same mechanisms that in nature are active during the phases of organogenesis, when all the stem cell differentiation processes that lead to the formation of tissues and organs take place. In these phases, where there is a high risk of developing errors in replication, differentiation factors play an important role in correcting those cells that are making mistakes. Reconfirmation of this mechanism occurred when, implanting cancer cells into an embryo during the organogenesis phase, differentiated and apoptotic processes were observed on implanted tumor cells. On the contrary, implanting tumor cells into an embryo after the organogenesis phase, tumor cells continued to proliferate. One can therefore talk of epigenetic reprogramming of diseased cells through the integration of those peptides that are able to restore the cell within its normal physiology.

These researches, begun in the late 1980s, from Biava and Coll (Cancer Letter 1988) and then have been developed by the Children's Hospital in Chicago, Northwestern University, La Sapienza University of Rome and other institutions. It emerges that differentiation factors are able, in association with standard chemotherapy treatments, to slow and often block the cell cycle of tumor cells either by activating in trascriptional way the p53 onco-repressor gene, and by regulating in post-translational way the protein of Retinoblastoma (pRb) which also has a block cell cycle activity. At the same time, a series of regulating gene cascades are also activated by the same differentiation factors as they attempt to repair cellular damage at the origin of malignancy: if the alterations are not too serious, they are effectively repaired; If mutations are too serious and are not repaired, the genes of programmed cell death / apoptosis are activated and the cancerous cells die.

In fact, after treatment with stem cell differentiation factors, tumor cells come out of the cellular multiplication cycle. A number of important researches on the synergy between chemotherapeutic agents and differentiation factors have already been carried out in 2011, for example, a research conducted at Current Pharmaceutical Biotechnology at La Sapienza University in Rome where in vitro the slowdown of Growth of CaCo2 colon cancer cells: a 35% slowdown was obtained by treatment with Fluoro-Uracil alone, a 98% slowdown was obtained with the simultaneous administration of 5-Fluoro-Uracyl and differentiation factors. The same Richard Ablin (PSA discoverer) reported in an article published in 2014 a greatly beneficial effect on the synergy between differentiation factors and surgical ablation or other traditional prostate cancer treatments. Stem cell differentiation factors also demonstrated improved performace staus and quality of life in 82% of patients treated and reduced adverse side effects of chemotherapy

The following are the main images related to the different studies carried out on embryo differentiation factors: The state of the art of research includes both in vitro works on molecular dynamics involving stem differentiation factors and in vivo work: on mice and on men. It remains necessary to implement in-vivo research, especially clinical. Specifically, studies have been conducted on the following aspects: -

Dowload and / or blocking of the cell cycle, activatin in trascriptional way the oncosupressor gene p53 ,

or regulating in post-traslational way the Retinoblastoma protein (pRb)

- Slower growth of tumor cell lines

- Animal studies

- Protein Analysis of Zebrafish Egg Extract

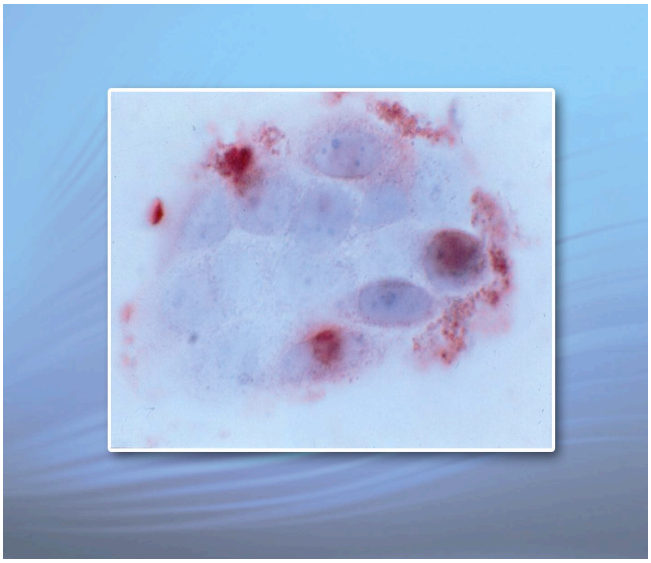
- Clinical study of 200 patients to evaluate possible side effects –

- Randomized clinical study in 179 patients with intermediate or advanced hepatocarcinoma .

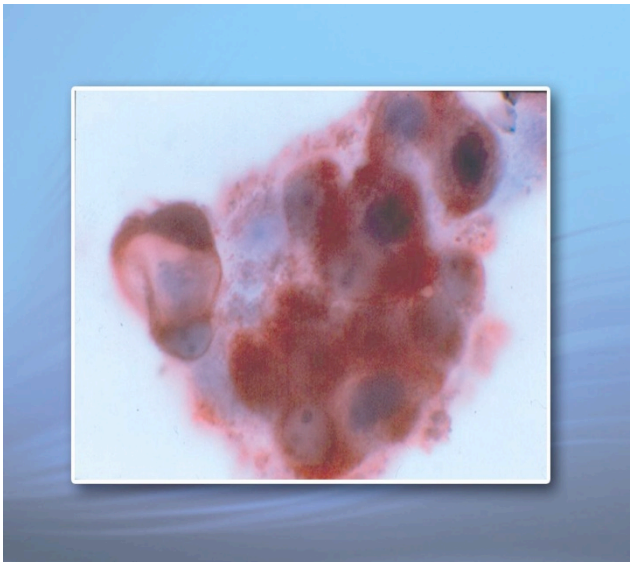
Download and / or block cell cycle action.

It has been shown that in the embryonic microenvironment there are factors that regulate the expression of the p53 co-repressor, activating it, and post-translationally pRb. In fact, with the differentiation of stem cell differentiating factors of different tumor lines, a block of cell cycle was observed in G1-S phase.

Through cytofluorometry and immunohistochemistry, a significant increase in the concentration of p53 protein has been demonstrated in specific lines of cellular tumor lines such as glioblastoma multiforme, melanoma and hepatocarcinoma treated with stem differentiation factors. This increase is a consequence of the transcriptional regulation of the p53 oncosoppress gene.



before the treatment



after the treatment

Biava, P.M.; Carluccio, A. *Activation of anti-oncogene p53 pro- duced by embryonic extracts in vitro tumor cells*. J. Tumor Marker Oncol., 1997, 12, 9-15

Post-traslational regulation of the Retinoblastoma protein.

By treating cancer cell lines such as those of kidney adenocarcinoma, a post-translational regulation of retinoblastoma protein (pRb) was observed, known for its cell cycle block function; This adjustment involves modification of the relationship between the phosphorylated form and the nonphosphorylated form of the pRb protein in favor of the non-phosphorylated form. This form blocks the cell cycle stopping the transcription of the E2F-1 gene.

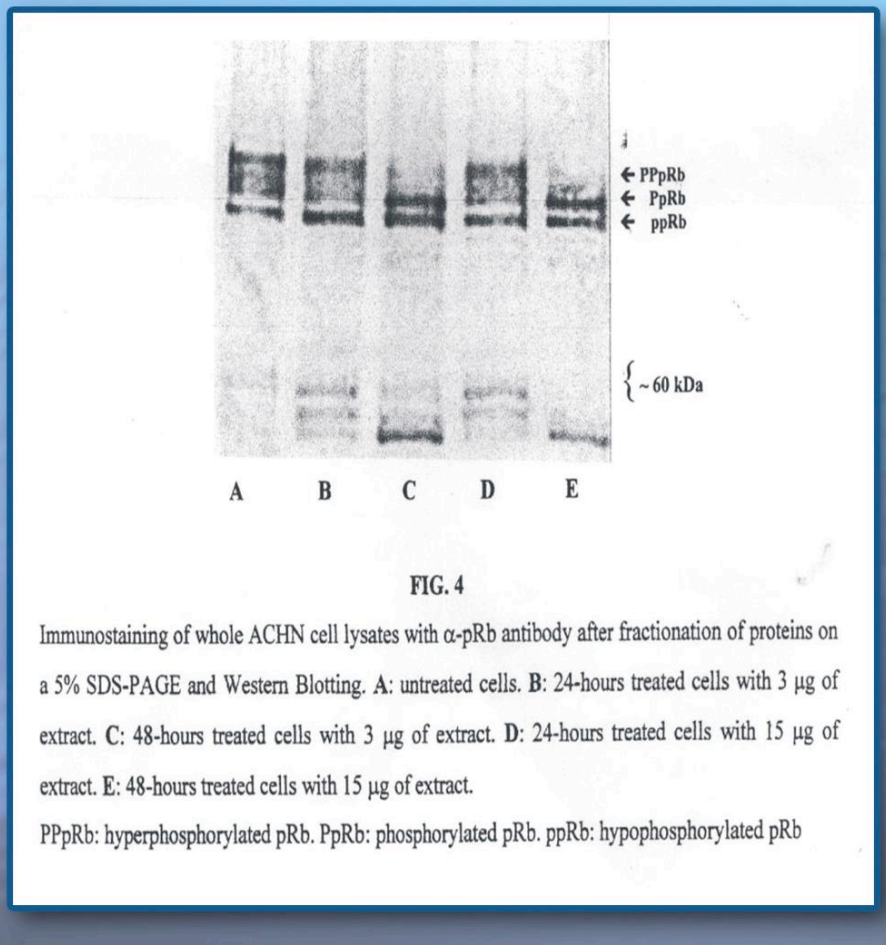


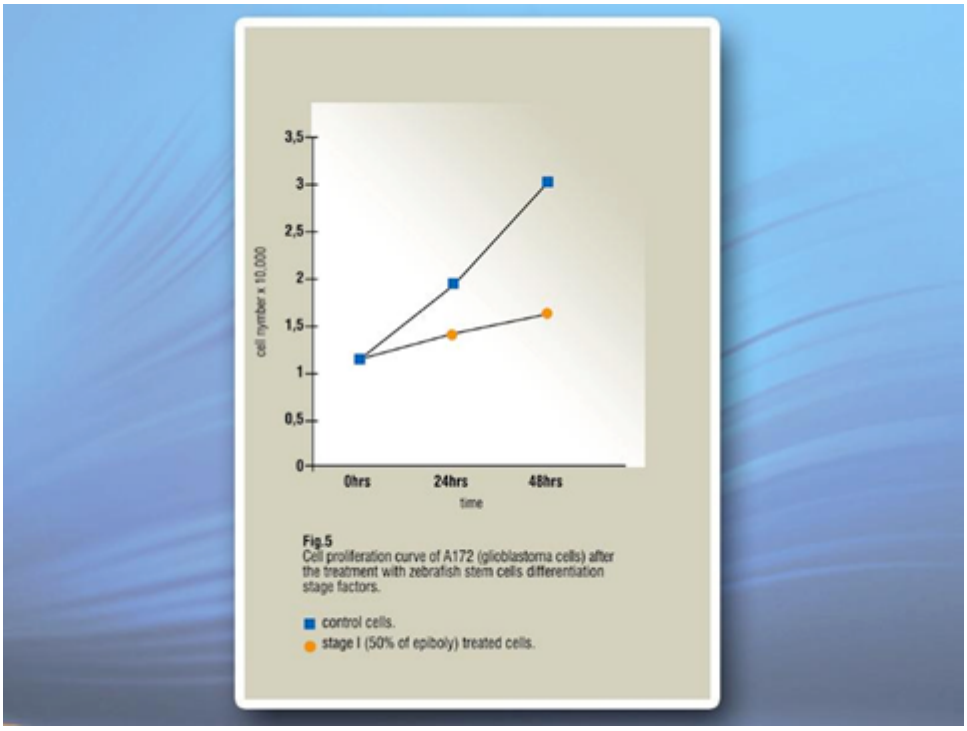
FIG. 4

Immunostaining of whole ACHN cell lysates with α-pRb antibody after fractionation of proteins on a 5% SDS-PAGE and Western Blotting. **A**: untreated cells. **B**: 24-hours treated cells with 3 µg of extract. **C**: 48-hours treated cells with 3 µg of extract. **D**: 24-hours treated cells with 15 µg of extract. **E**: 48-hours treated cells with 15 µg of extract.

PPpRb: hyperphosphorylated pRb. PpRb: phosphorylated pRb. ppRb: hypophosphorylated pRb

Biava, P.M.; Bonsignorio, D.; Hoxa, M.; Facco, R.; Ielapi, T.; Frati, L.; Bizzarri, M. Post-traslational modification of the retino-blastoma protein (pRb) induced by in vitro administration of Ze- brafish embryonic extracts on human kidney adenocarcinomacell line. J. Tumor Marker Oncol., 2002, 17, 59-64

Slow down the growth of tumor cell lines .
These blocking mechanisms of the cell cycle have been observed in several tumor cell lines. Specifically, tumor cell lines were investigated of: - Glioblastoma - Melanoma - Breast cancer - Lymphoblastic Leukemia - kidney adenocarcinoma .



Glioblastoma (Biava et al)

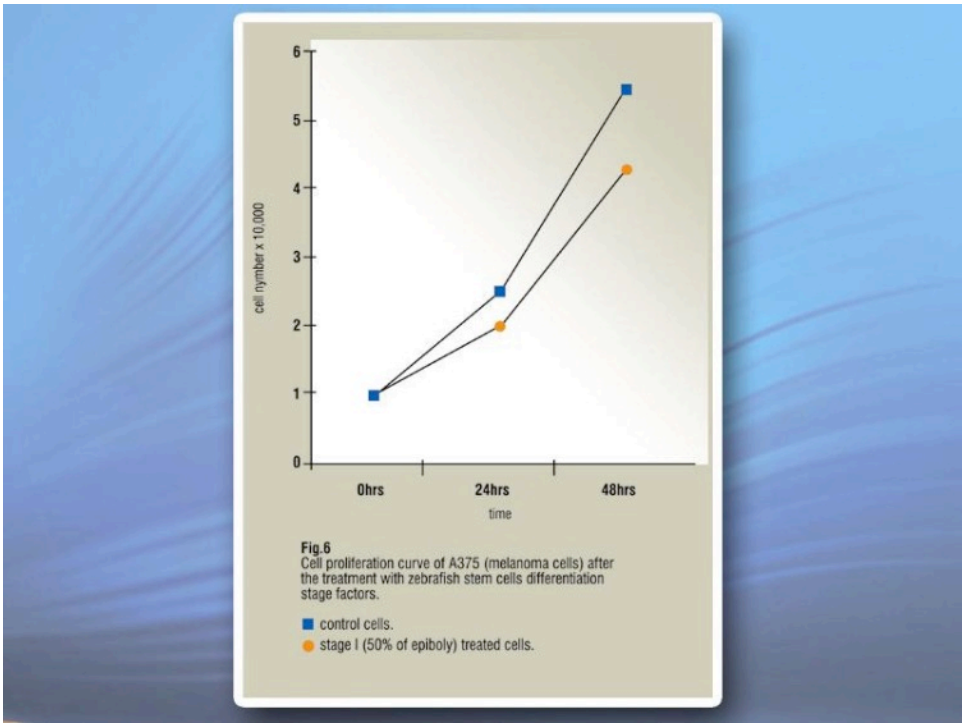


Fig.6
Cell proliferation curve of A375 (melanoma cells) after the treatment with zebrafish stem cells differentiation stage factors.

■ control cells.
● stage I (50% of epiboly) treated cells.

Melanoma (Biava et al.)

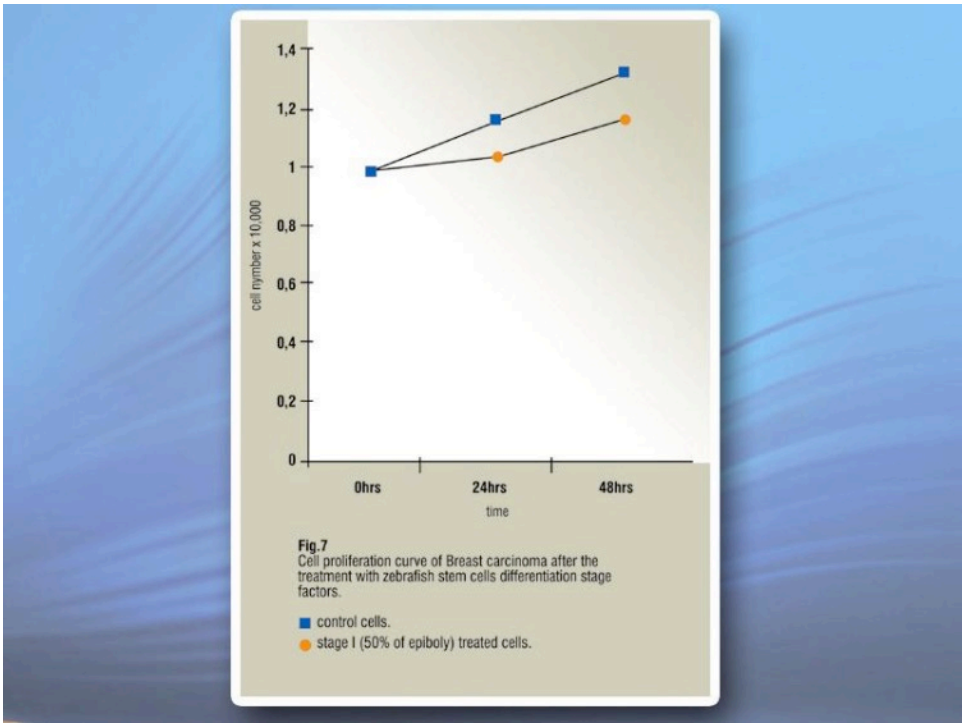
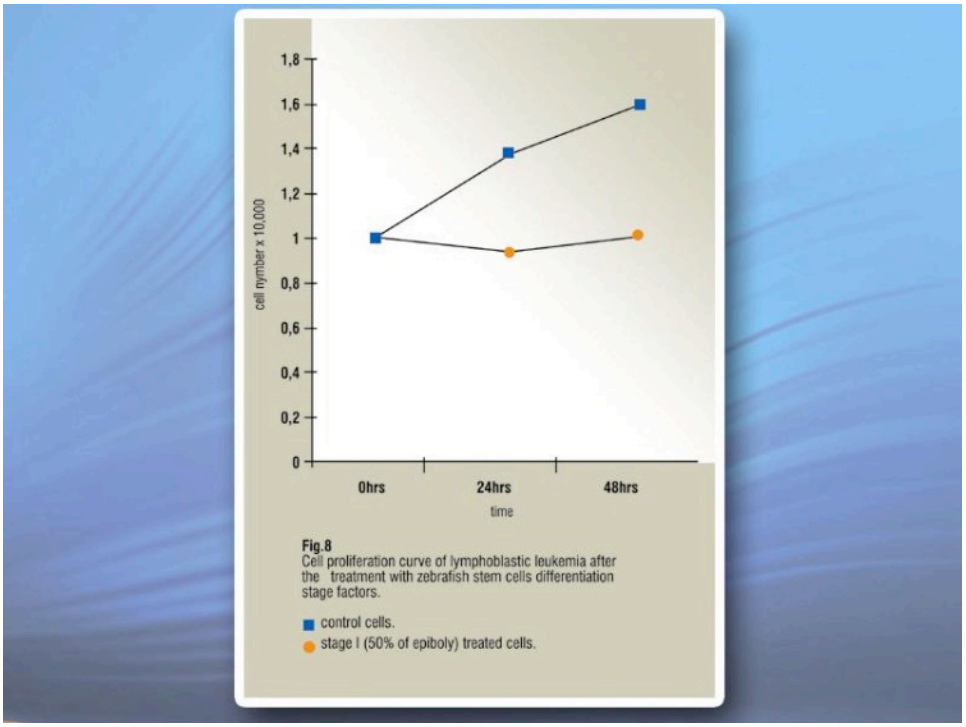


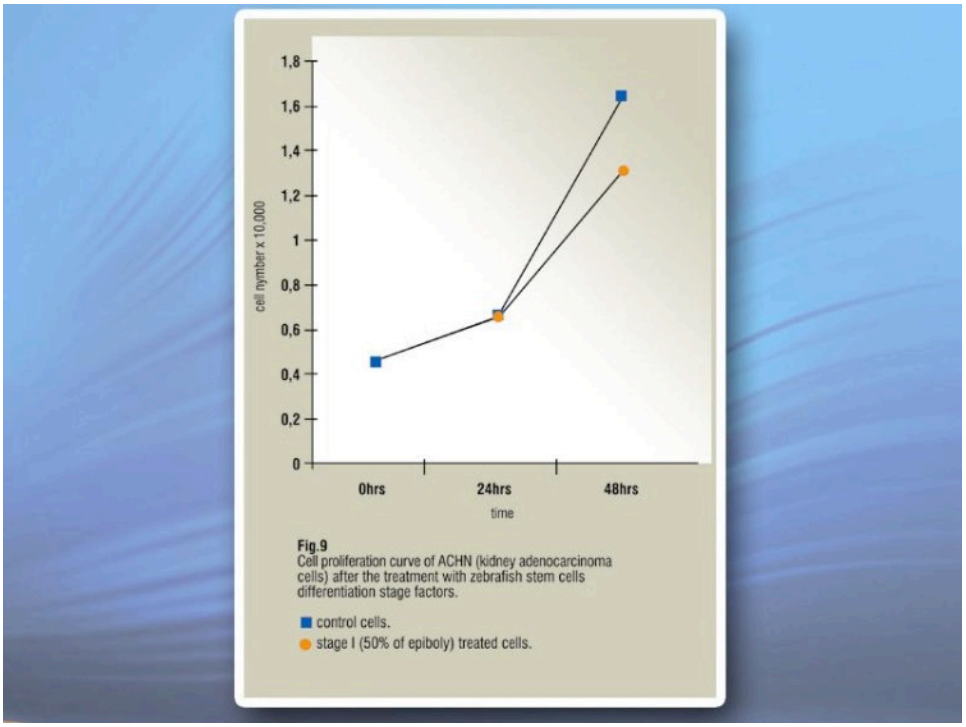
Fig.7
Cell proliferation curve of Breast carcinoma after the treatment with zebrafish stem cells differentiation stage factors.

■ control cells.
● stage I (50% of epiboly) treated cells.

Breast cancer (Biava et al.)



Acute Lymphoblastic Leukemia (Biava et al)



Kidney adenicarcunoma (Biava et al)

Animal Studies

Animal studies.
The effects of stem differentiation factors on inhibition of tumor growth were in vivo tested on females of singular C57BL / 6 mice from the weight of 18-20 grams to which a subcutaneous Lewis primary carcinoma injection was performed . Therefore, both the size of the primary tumor, and the survival time of the mice, have been evaluated. In terms of development of the primary tumor, an extremely significant difference (P <0.001) was observed between treated and control mice (Figure 1) and thus also with regard to the survival ratio, always in favor of the treated mouse.

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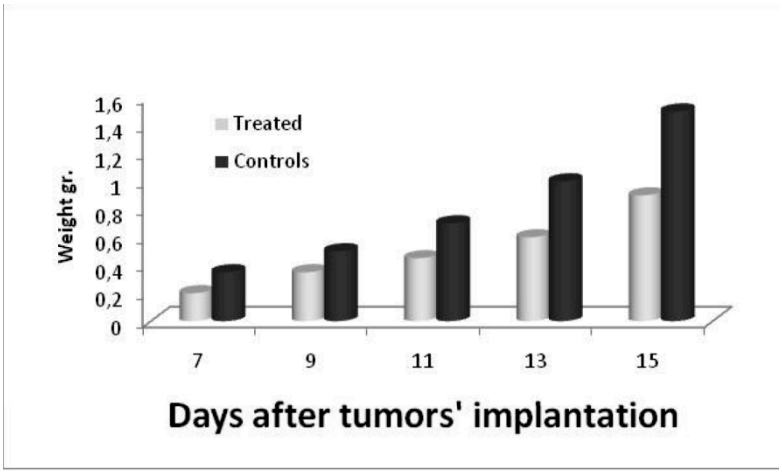
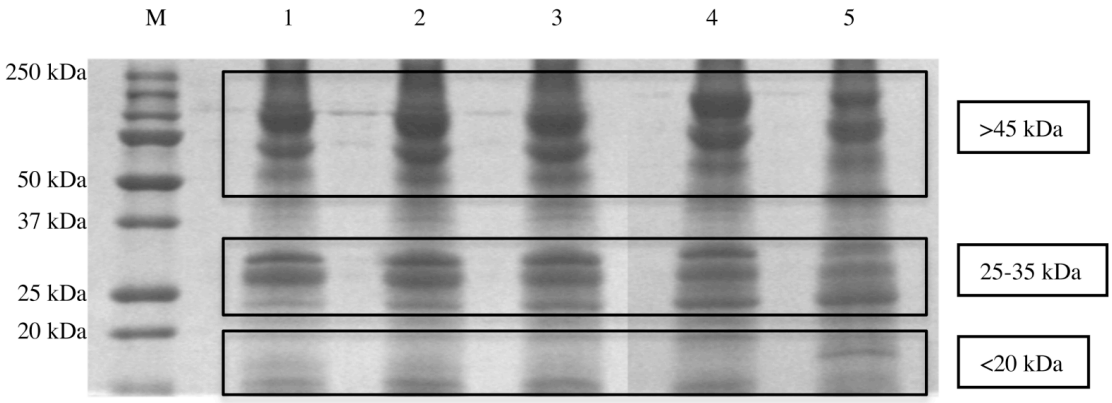
Fig. 1



Biava, P.M.; Bonsignorio, D.; Hoxha, M.; Impagliazzo, M.; Frosi, A.; Larese, F.; Negro, C. (2002) *Mother-embryo cross-talk: the anti-cancer substances produced by mother and embryo during cell differentiation. A review of experimental data*. J. Tumor Marker Oncol., 2002, 17, 55-58

Protein Analysis of Zebrafish Egg Extract:

A protein analysis of embryonic extract from zebrafish eggs was performed. A suspension of the glycerol-alcohol solution was analyzed with monodimensional gel electrophoresis (SDS-PAGE). As shown in the figure below in all 5 phases extracted, they can be distinguished by their molecular weight by three major groups: over 45 kDa, about 25-35 kDa and below 20 kDa. In any case, the relative amount of protein is different in the 5-stage samples.



Below is the list of proteins that have been identified by Biava et al.with the mass spectrometry analysis. The asterisk (*) lists the proteins that have never been described in the Zebrafish embryo

Table 1
List of protein identified using the nano LC-ESI-Q-TOF in Zebrafish embryo at midle-blastula-gastrula stage

Accession	Protein Name	Score	Molecular Waight	pI calculated	Sequence coverage
gi|166795887	vitellogenin 1 precursor	1108	150308	8,68	19
gi|94733730	vitellogenin 1	1039	149825	8,74	21
gi|94733733	novel protein similar to vitellogenin 1 (vg1)	913	149828	8,92	19
gi|94733734	novel protein similar to vitellogenin 1 (vg1)	835	150550	8,83	16
gi|145337918	Vtg1 protein	780	116965	9,07	18
gi|94733731	novel protein similar to vitellogenin 1 (vg1)	762	149911	8,84	19
gi|94732723	novel protein similar to vitellogenin 1 (vg1)	745	147826	8,73	17
gi|159155252*	Zgc:136383 protein	720	124413	8,78	17
gi|68448530	vitellogenin 5	559	149609	8,77	13
gi|92097636	Zgc:136383	402	28924	9,33	36
gi|63100501	Vtg1 protein	345	36580	9,23	28
gi|57864789	vitellogenin 7	341	24490	8,37	40
gi|57864783	vitellogenin 4	334	31304	9,48	27
gi|113678458	vitellogenin 2 isoform 1 precursor	323	181208	8,70	11
gi|125857991	Zgc:136383 protein	171	149328	8,93	9
gi|15209312*	procollagen type I alpha 2 chain	169	147826	9,35	4
gi|57864779	vitellogenin 2	122	69906	7,84	8
gi|11118642	vitellogenin 3 precursor	117	140477	6,92	2
gi|303227889	vitellogenin 6	73	151677	8,84	4

Continues

Accession	Protein Name	Score	Molecular Waight	pI calculated	Sequence coverage
gi|13242157 *	egg envelope protein ZP2 variant A	71	48194	6,04	5
gi|6644111 *	nucleoside diphosphate kinase-Z1	69	17397	7,77	14
gi|18859071*	nucleoside diphosphate kinase 3	69	19558	7,68	7
gi|126632622*	novel prot. cont. a galactose binding lectin domain	67	19245	9,33	13
gi|66773080 *	mitochondrial ATP synthase beta subunit-like	66	55080	5,25	4
gi|38541767*	Ppia protein	60	19745	9,30	13
gi|1865782	HSC70 protein	58	71473	5,18	2
gi|28279108	heat shock protein 8	58	71382	5,32	4
gi|41152402*	histone H2B 3	49	13940	10,31	11
gi|41393113*	collagen, type I, alpha 1b precursor	46	137815	5,39	4
gi|94732492 *	ras homolog gene family, member F	46	24035	9,00	6
gi|47778620 *	tryptophan hydroxylase D2	45	55686	6,56	1
gi|68448517 *	zona pellucida glycoprotein 3.2 precursor	44	47365	4,92	2
gi|326677766 *	PREDICTED: RIMS-binding protein 2-like	41	138659	5,86	0
gi|112419298	Vtg3 protein	40	60622	6,32	2
gi|54400406 *	glutaredoxin 3	39	36541	5,18	11
gi|41152400*	peptidylprolyl isomerase A, like	37	17763	8,26	7

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 36 indicate identity or extensive homology ($p<0.05$).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

Clinical studies:

Two clinical studies have been carried out to first check the safety and then the effectiveness of integration with the stem differentiation factors of standard chemotherapy treatments. A first study was conducted on 200 patients, including 60 with advanced breast cancer, to evaluate possible side effects: for this reason no control group was foreseen. The protocol included administering patients 1 ml 3 times a day as sublingual drops containing 3 microgram / ml staminal differentiation factors. After 3 years of treatment, no adverse events were reported in any of the 200 patients treated; In addition, 80% of these patients showed an improvement in the performance status, evaluated with the E.C.O.G scale: generally, the status status shifted from a 4 to 3 state to one of 2 or 1. In the 60 breast cancer patients there were also 4 cases of partial regression and 70% of survival after 3 years. Biava, P.M .; Bonsignorio, D .; Impagliazzo, M .; Frosi, A .; Larese, F .; Negro, C .; Hermann, G.F .; Matarese, S .; Malzac, J .; Pontiggia, P., & lt; / RTI & gt; Embryonic differentiation factors with anti-cancer properties: preliminary clinical results in the therapy for advanced tumors. J. Tumor Marker Oncol., 2002, 17 (3), 65-69 A randomized clinical trial was performed on 179 patients with intermediate or advanced hepatocarcinoma. The results showed a statistically significant difference between the group treated with the synergy of differentiation factors. A randomized clinical trial was performed on 179 patients with intermediate or advanced hepatocarcinoma. The results showed a statistically significant difference between the group treated with the synergy of differentiation factors and standard treatments and the control group (P = 0.03), a difference in favor of the group treated with integration. There were 19.8% of regression (2.5% of which total regression) and 16% of disease stabilization. Livraghi, T .; Meloni, F .; Frosi, A .; Lazzaroni, S .; Bizzarri, M .; Frati, L .; Biava, P.M. Treatment with stem cell differentiation stage factors of intermediate-advanced hepatocellular carcinoma: an open randomized clinical trial. Oncol. Res., 2005, 15, 399-408

Conclusions:

The committee met to evaluate the scientific solidity of the researches described confirmed the importance of this course of study and encouraged at the university level the development of further research both on an animal model and at a clinical level. There is also the possibility of developing supplements foods that inspire these researches to provide physicians with a valuable support to integrate much of the differentiation factors described in the literature. Such solutions are to be understood only as integration into standard therapies and it is hoped that they can be validated in advance by appropriate clinical trials.