THE ZEBRAFISH EMBRYO DERIVATIVE AFFECTS CELL VIABILITY OF EPIDERMAL CELLS: A POSSIBLE ROLE IN THE TREATMENT OF PSORIASIS

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The zebrafish embryo derivative affects cell viability of epidermal cells: a possible role in the treatment of psoriasis

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In patients affected by psoriasis, use of a topical formula containing a derivative of zebrafish embryos was associated with reduced skin inflammation and dermal turnover, as well as a generally better outcome. In an attempt to understand the molecular mechanisms lying beyond these findings, we investigated the anti-proliferative effects of the zebrafish embryos derivative by addressing the mitochondrial function (MTT assay) and cell nuclei distribution (Hoestch staining). In cell cultures stimulated with fetal calf serum (FCS) or epidermal growth factor (EGF), the zebrafish derivative significantly inhibited cell proliferation induced by either approach, although the effect was stronger in cells stimulated with FCS. These results suggest that the zebrafish embryos derivative may dampen increased cell proliferation; this observation may be relevant to cutaneous pathologies related to altered proliferative mechanisms, including psoriasis.

**KEY WORDS:** Psoriasis - Zebrafish - Cell proliferation - Epidermal growth factors.

Psoriasis is a relapsing, non-infective, autoimmune chronic form of dermatitis (affecting the scalp, elbows, knees, lumbosacral region, genital skin, soles of the feet and palms) that is extremely common worldwide. The most usual dermatologic events are papules and clearly defined erythematous papules that can develop erythematous-desquamative plaques.1

From a histological point of view, psoriatic lesions appear as skin hyper-proliferation areas - whose epidermal turnover is 5 to 10 times faster than that of the normal skin - accompanied by incomplete maturation of keratinocytes and the retention of nuclei in the stratum corneum (parakeratosis), with neovascularisation, increased blood flow, protein-rich exudate and limited capabilities of the lymphatic vessels. Also noticeable is the inflammatory response supported by the infiltration of polymorphic neutrophils in the skin.2

The causes of psoriasis are still unknown, and the available data seems to hint at a multi-factorial origin that certainly involves genetics and autoimmunity. In any case, most authors agree that the most important cause of the problem is an altered Th1-mediated immune response, consequent to a previous inflammatory insult. Whatever the triggering factor, the pool of triggered T lymphocytes causes the release of different active substances (gamma-interferon, lymphokines, growth factors).

However, these do not seem to be the only substances responsible for the reaction. As a matter of fact, psoriatic skin shows high levels of NGF, β-endorphin and other angiogenic factors, as well as a local reactivity with reduced cell apoptosis.3-6

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A preparation for topical use was recently developed and successfully tested in clinical trials. It contains some active ingredients well known for their anti-inflammatory (18-beta glycyrrhetic acid phytosome, boswellic acids, Zanthoxyllum bungeanum extract) and anti-psoriatic properties (7-dehydrocholesterol), together with a particular peptide derivative obtained from zebrafish (Brachydanio rerio) embryos. This derivative, containing low-molecular-weight protein factors (15 KDa) - obtained through homogenisation and subsequent filtration and purification - seems to be able to control some phases of the cell cycle with the stabilisation of the differentiation processes. This action was demonstrated in vitro in tests intended to assay the mitotic proliferation of keratinocyte clones. This action appeared to be particularly beneficial in patients affected by psoriasis, where the reduced capability of cell differentiation of the basal lamina causes plaque formation to be reduced owing to a lower proliferative index of keratinocytes.

The study was thus aimed at verifying whether the effect that had previously been observed with the peptide derivative obtained from zebrafish embryos might be also linked to the anti-proliferative action of the compound. For this reason the derivative obtained from zebrafish embryos was tested in cell cultures following induction of cell proliferation through administration of fetal calf serum (FCS) or epidermal growth factor (EGF).

Materials and methods

The study of the anti-proliferative effect of the zebrafish embryo derivative on epidermal cells (Keratinocytes, Life Technologies, USA, maintained in medium 154) was conducted using a mitochondrial function assay with methyl tetrazolium (MTT assay) purchased from Sigma (USA). The 12-well culture plates (6x10^4 cells per well) used for the test contained FCS or EGF in the presence or absence of the zebrafish derivative. Cell viability was determined as the ratio between the cells incubated with the zebrafish derivative and the cells incubated without it (control). The MTT assay was conducted according to a model described in a previous work.

Viable and actively proliferating cells retain an important mitochondrial activity, and this aspect is exploited by incubating the cells (treated in the above different experimental conditions) with the compound in question, which is then metabolised by mitochondria and produces a coloured salt. The latter is solubilised in isopropanol and transferred into a plate reader. The signal intensity is a function of the amount of viable cells found in the well. According to the adopted experimental pattern, FCS (10%, Euroclone, Italy), or EGF (1 ng/mL; Gibco, USA) or the vehicle alone (RPMI 1640) were used as proliferative stimuli.

Since the zebrafish derivative contains 60% glycercol and 5% ethanol, the same quantity of excipients was added to final volume of the “control” sample.

The zebrafish derivative concentration used in the experiments amounted to 10µg/mL. In the case of treatment with FCS as the proliferative stimulus, a fluorescence analysis was also performed in order to highlight the cell nuclei by Hoechst staining. The fluorescence analysis was performed according to a method described in a previous work.

The information provided by the text and figures is expressed as the mean value ± standard deviation and refers to 4 different experiments. The statistical analysis was performed using the ANOVA test, followed by the Bonferroni analysis using SPSS 18.0 software.

Results

The increase in cell viability following FCS stimulation (Figure 1) was significantly reduced in the presence of the compound (obtained by mixing the four chief stages of the embryo development process, which are generally linked to different gene expression profiles), while the reduction was lower when the cells were incubated with the individual stages.

When looking at Figure 1 it can be seen that cell viability decreases at 96 hours, and this is probably the sign of some exhaustion of the cell growth potential in the presence of FCS.

The detailed data concerning 72 hours stimulus (Figure 2) shows a large and statistically significant reduction due to treatment with the zebrafish derivative containing all the four embryo stages.

In the case of treatment with FCS as the proliferative stimulus, a fluorescence analysis was also performed in order to highlight the cell nuclei through Hoechst staining.
Figure 1.—Cell viability in the presence of FCS and zebrafish embryo derivative. Mean ± standard error is shown; *P<0.05 vs. FCS; **P<0.01 vs. FCS.

Figure 2.—Cell viability test at 72 hours following stimulation with FCS. Mean ± standard error is shown; *P<0.05 vs. FCS; **P<0.01 vs. FCS.

Figure 3.—Immunofluorescence through Hoechst staining.

Figure 3 clearly shows a reduction in the cells of the samples treated with the zebrafish derivative, particularly in those treated with the derivative containing all the embryo stages.

Treatment with EGF alone was found to be less efficient in inducing cell proliferation, but even in this experimental condition (Figure 4) the zebrafish derivative containing all the four stages proved to be the only remedy capable of slowing down cell proliferation.

The detailed data concerning 72 hours proliferative stimulus (Figure 5) shows a statistically significant reduction due to treatment with the zebrafish derivative containing all the four stages.
Discussion

The aim of this work was to investigate the effect of the derivative obtained from zebrafish embryos on cell viability in proliferating keratinocytes.

On the basis of the resulting experimental data it is possible to observe how the compound shows a good anti-proliferative action highlighted by both the MTT assay and nuclear analysis through fluorescence. Both the extract prepared during the first stage (1) and that prepared during the last stage (4) of the embryo growth process demonstrated to possess some antiproliferative capability; nevertheless the derivative containing all the four stages (mix) proved to be more effective with a statistically significant activity.

This result was observed following stimulation with either FCS or EGF. It may be hypothesised that the higher efficacy recorded with FCS is due to the presence of different growth factors and the possibility that the extract containing the four different stages may have a more homogeneous composition that is capable of interfering with different mechanisms associated with cell proliferation. Future studies are warranted to investigate the zebrafish embryo derivative effects in cells under psoriatic pathological conditions.

These results agree with the observation that the zebrafish embryo derivative contained in the topical formulation is effective in counteracting psoriasis symptoms;7,8 while also hinting at the possibility that, in addition to the antiphlogistic activity observed in the preparation and ascribable in particular to its content in glycyrrhetic acid and boswellieic acids, the preparation may relieve skin symptoms also through modulation of the cell turnover.

The preliminary indications of this study offer a scientific rationale for the use of the zebrafish embryo derivative in preparations whose therapeutic target is the control of the skin cell turnover with particular reference to possible dermatological applications.

Riassunto

Il derivato di embrioni da zebra fish influenza la viabilità cellulare delle cellule epidermiche: possibile ruolo nel trattamento della psoriasi

L'utilizzo topico di una preparazione contenente un derivato di embrioni da zebra fish ha dimostrato, in pazienti psoriasici, di poter ridurre la sintomatologia, l'inflammazione cutanea e il turnover epidermico. Per capire se questi meccanismi potessero essere legati al controllo della crescita cellulare, si è deciso di investigare l'effetto antiproliferativo di tale derivato sulle cellule di origine epidermica attraverso l'analisi della vitalità cellulare mediante la valutazione della funzionalità mitocondriale (MTT) e della distribuzione nucleare (colorazione di Hoestech). Il derivato embrionale di zebra fish è risultato efficace nel contrastare la proliferazione cellulare in colture cellulari stimolate con fetale cile serum (FCS) o epidermal growth factor (EGF). Il dato è apparso particolarmente evidente nei saggi con FCS, in cui l'effetto proliferativo risultato...
essere la conseguenza della stimolazione da parte di differenti fattori di crescita. I risultati ottenuti suggeriscono un possibile ruolo del derivato da zebrafris nel controllare l'aumentata risposta proliferativa tipica dell'epidermoide di pazienti psoriasici.

**Parole chiave:** Psoriasi - Zebrafris - Cellule, proliferazione - Fattore di crescita epidermica.

**References**


**Conflicts of interest.**—Dr. Biava owns a patent related to the zebrafris embryo. He is also the president of the Novacell Biotech Company, which owns the "Staminbio" brand.

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