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Research Article

Exploring The Effects Of Secretome From Wharton's Jelly Mesenchymal Stem Cells In Zebrafish

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ABSTRACT

Cell-free therapy based on conditioned media derived from mesenchymal stem cells has gained attention in the field of protective and regenerative medicine, due to their high content of growth, trophic and protective factors. However, the exact composition and properties of conditioned media can vary greatly depending on multiple parameters, so that the evaluation in vivo of the effects and biosafety of these products is essential. Our recent efforts have been focused on the optimization of a procedure for high-efficient preparation of toxic-free conditioned medium derived from Wharton's jelly mesenchymal stem cells. Moreover, by a multiparametric analysis in zebrafish, we showed that exposure to our conditioned medium preparations triggers antioxidant, anti-apoptotic and pro-regenerative effects. Building on this evidence, here I report detailed morphometric analysis showing that morphological parameters of developing zebrafish embryos were largely unaffected by exposure to conditioned medium, thus confirming the biosafety in vivo of our conditioned medium preparations. This information represents a further step towards future therapeutic application of our conditioned medium preparation.

Index Terms- secretome, Wharton's jelly, mesenchymal stem cells, zebrafish.

I. INTRODUCTION

Several studies and clinical trials in the field of regenerative medicine have suggested that the benefits of mesenchymal stem cell transplantation are a direct result of the paracrine effect elicited by a diverse array of bioactive molecules released by these cells, commonly known as the secretome or conditioned medium [1]-[5]. Notably, the conditioned medium produced during the in vitro culturing of mesenchymal stem cells can be collected and used as an excellent source of secretory bioproducts for cell-free therapeutic applications [6]. The primary advantage of such a therapeutic approach lies in the substantial enhancement of patient safety, achieved through circumventing ethical constrains and mitigating adverse events typically associated with cell transplantation [6]. On the other hand, several studies revealed that the qualitative and quantitative composition, and consequently the biological efficacy, of conditioned media are heavily affected by a excessive variability in stem cell sources and manufacturing methods [7]-[8]. To address this limitation, a recent study conducted from my research group described an optimized procedure for preparation of highly reproducible conditioned medium samples from Wharton's jelly mesenchymal stem cells isolated from enzymatic-free explant culturing of umbilical cord fragments [9]. Worth mentioning, we observed no substantial differences in the capacity of cells from different donors to produce conditioned medium samples of comparable compositional analysis, when they are isolated, grown and induced, under the standardized conditions outlined in our protocol [9].
Aquatic organisms provide an ideal platform for high-throughput toxicological analysis [10]-[12]. In particular, we used zebrafish to explore the biosafety and effects elicited by our standardized conditioned medium preparations. Through a multiparametric analysis, we showed that exposure of developing zebrafish embryos to conditioned medium triggers antioxidant, pro-survival and pro-regenerative effects, impinging on

specific marker gene expression [13].
Herein, I report a detailed morphometric analysis showing that morphological parameters of developing zebrafish embryos were largely unaffected by exposure to conditioned medium, thus confirming the in vivo biosafety of our conditioned medium preparations.

II. MATERIALS AND METHODS

Zebrafish maintenance, embryo exposure to conditioned medium and morphometric analysis
The Zebrafish Laboratory of the Advanced Technologies Network Center at the University of Palermo is allowed to use zebrafish for scientific research by the Italian Ministry of Health (aut. prot. no. 24/2023-UT dated 13 June 2023) and by the Provincial Health Authority of Palermo (code no. 053PA414 dated 6 June 2019). Wild-type (AB strain) zebrafish adults were maintained in a recirculating aquaculture system (Tecniplast) under standard conditions, according to National (Italian D.lgs 26/2014) and European (2010/63/EU) animal welfare laws.
Viable and synchronously developing embryos were obtained as described [13], placed in 96-well culture plates (1 embryo/well), kept at 28.5±0.5°C, and exposed continuously from 6 to 72 hours post-fertilization (hpf) to E3 medium (5 mM NaCl, 0.33 mM CaCl2, 0.17 mM KCl, 0.33 mM MgSO4) containing conditioned medium at 75 µg/mL (referred to the total protein content of CM samples). Control groups for these experiments included sibling embryos exposed to standard E3 medium. Treated and control embryos at 72 hpf were carefully examined by observation under a M205-FA multidimensional stereomicroscope (Leica Microsystems, Milan, Italy). Digital images were captured and the ImageJ software was used to assess morphometric parameters in 50 larvae per each experimental group.
All the experimental protocols described in this study were carried out exclusively with zebrafish embryos and larvae up to 72 hpf. At this stage of their life cycle, zebrafish are not capable of independent feeding and therefore are not subjected to the Italian (D.lgs 26/2014) and European (2010/63/EU) rules on the protection of animals used for scientific purposes.
The criteria of normality of data and homogeneity of variance were assessed by using the Kolmogorov–Smirnov test and Levene's test, respectively. For normally distributed data, one-way ANOVA analysis followed by Tukey HSD test was performed. Otherwise, non-parametric Kruskal–Wallis was used. The differences among test groups were assessed to be significant at $p < 0.05$.

III. RESULTS AND DISCUSSION

Morphometric analysis was performed on developing zebrafish embryos exposed to conditioned medium at 75 µg/mL. Preliminary experiments suggested that zebrafish embryogenesis was not altered at this dosage, with the vast majority of embryos exposed to conditioned medium exhibiting survival and hatching rates comparable to those of control groups reared in standard E3 medium [13].
Leveraging this evidence, we performed a detailed morphometric analysis in control and treated larvae at 72 hpf, a stage at which tissue-specific epigenetic and transcriptional programs have been established [11], [14]-[16]. We measured the following morphometric parameters: body length, yolk diameter, pericardic area, head width, eye length, eye width, interocular distance, and body pigmentation. Each measurement was repeated three times, averaged for statistical analysis, and normalized to that of control larvae. Worth mentioning, the ratio between the values of each morphometric parameter in treated and control groups remained largely unchanged, indicating no statistically relevant difference ($p > 0.05$) between control and treated larvae at 72 hpf (Figure 1).

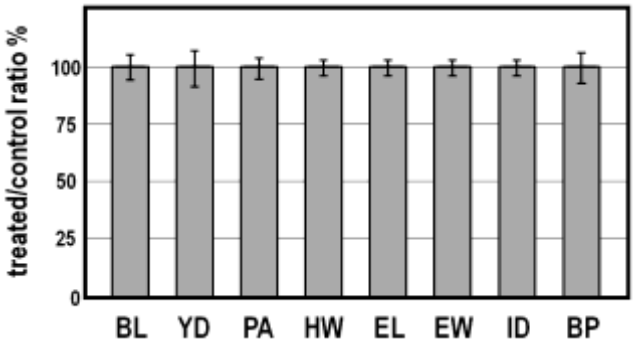

Figure 1. Morphometric analysis performed on zebrafish control and conditioned medium-treated larvae at 72 hpf. Each column in the bar chart indicate a morphological parameter control E3 groups, while blue columns indicate the ratio between treated and control averaged values measured for each morphometric parameter. Abbreviations of the examined parameters: BL, body length; YD, yolk diameter; PA, pericardic area; HW, head width; EL, eye length; EW, eye width; ID, interocular distance; BP, body pigmentation.

Altogether, these results are in strict concordance with previous reports [13], and further support the evidence that our conditioned medium preparations did not inflict toxicity in vivo at the dosage of 75 µg/mL.
Most probably, the absence of morphological variation in treated larvae reflects similar epigenetic states at the loci governing embryogenesis and tissue-specific differentiation processes. In fact, it is widely accepted that the epigenome can act as the link between environmental cues, both external and internal, to the organism and phenotype by converting the environmental stimuli to phenotypic responses through changes of gene transcription outcomes [17]-[21]. This aspect warrants further investigation by means of chromatin immunoprecipitation analysis [22]-[23].
At this stage, the encouraging findings described in this paper represent a further step towards future therapeutic application of our conditioned medium preparation.

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REFERENCES

1. M. Gnecchi, H. He, N. Noiseux, O. D. Liang, L. Zhang, F. Morello, et al., “Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement,” FASEB J., 20, pp. 661–669, 2006.
2. L. Chen, E. E. Tredget, P. Y. Wu, Y., Wu, “Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing,” PLoS ONE, 3, e1886, 2008.
3. S. H. Lee, S. Y. Jin, J. S. Song, K. K. Seo, K. H. Cho, “Paracrine effects of adipose-derived stem cells on keratinocytes and dermal fibroblasts,” Ann. Dermatol., 24, pp. 136–143, 2012.
4. M. Chudickova, I. Vackova, L. Machova Urdzikova, P. Jancova, K. Kekulova, M. Rehorova, et al, “The Effect of Wharton Jelly-Derived Mesenchymal Stromal Cells and Their Conditioned Media in the Treatment of a Rat Spinal Cord Injury,” Int. J. Mol. Sci., 20, 4516, 2019.
5. A. Joseph, I. Baiju, I. A. Bhat, S. Pandey, M. Bharti, M. Verma, et al, “Mesenchymal stem cell-conditioned media: A novel alternative of stem cell therapy for quality wound healing,” J. Cell Physiol., 235, pp. 5555–5569, 2020.
6. T. N. A. Gunawardena, M. T. Rahman, B. J. J. Abdullah, N. H. Abu Kasim, “Conditioned media derived from mesenchymal stem cell cultures: The next generation for regenerative medicine,” J. Tissue Eng. Regen. Med., 13, pp. 569–586, 2019.
7. A. Clabaut, C. Grare, T. Léger, P. Hardouin, O. Broux, “Variations of secretome profiles according to conditioned medium preparation: the example of human mesenchymal stem cell-derived adipocytes,” Electrophoresis, 36 (20), pp. 2587–2593, 2015.
8. B. Lukomska, L. Stanaszek, E. Zuba-Surma, P. Legosz, S. Sarzynska, K. Drela, “Challenges and controversies in human mesenchymal stem cell therapy,” Stem Cells Int., 9628536, 2019.
9. S. Acuto, M. Lo Iacono, E. Baiamonte, R. Lo Re, A. Maggio, V. Cavalieri, “An optimized procedure for preparation of conditioned medium from Wharton's jelly mesenchymal stromal cells isolated from umbilical cord,” Front. Mol. Biosci., Oct 2;10:1273814, 2023.
10. V. Cavalieri, “Model organisms and their application in environmental epigenetics”, In Environmental Epigenetics in Toxicology and Public Health; Fry, R., Ed.; Elsevier: Amsterdam, The Netherlands, pp. 67–87, 2020.
11. V. Cavalieri, G. Spinelli, “Environmental epigenetics in zebrafish,” Epigenetics Chromatin, Oct 5;10(1):46, 2017.
12. M. Pellicanò, P. Picone, V. Cavalieri, R. Carrotta, G. Spinelli, M. Di Carlo, “The sea urchin embryo: a model to study Alzheimer's beta amyloid induced toxicity,” Arch. Biochem. Biophys., Mar 1;483(1), pp. 120-126, 2009.
13. C. Reina, C. Cardella, M. Lo Pinto, G. Pucci, S. Acuto, A. Maggio, et al, “Antioxidant, Pro-Survival and Pro-Regenerative Effects of Conditioned Medium from Wharton's Jelly Mesenchymal Stem Cells on Developing Zebrafish Embryos,” Int. J. Mol. Sci., Aug 25;24(17):13191, 2023.
14. S. Balasubramanian, A. Raghunath, E. Perumal, “Role of epigenetics in zebrafish development,” Gene, 718, 144049, 2019.
15. V. Cavalieri, “Histones, Their Variants and Post-translational Modifications in Zebrafish Development,” Front. Cell. Dev. Biol., Jun 5;8:456, 2020.
16. V. Cavalieri, “The Expanding Constellation of Histone Post-Translational Modifications in the Epigenetic Landscape,” Genes (Basel), Oct 10;12(10):1596, 2021.
17. V. Di Caro, V, Cavalieri, R. Melfi, G. Spinelli, “Constitutive promoter occupancy by the MBF-1 activator and chromatin modification of the developmental regulated sea urchin alpha-H2A histone gene,” J. Mol. Biol., Feb 2;365(5), pp. 1285-1297, 2007.

18. V. Cavalieri, R. Melfi, G. Spinelli, “Promoter activity of the sea urchin (Paracentrotus lividus) nucleosomal H3 and H2A and linker H1 {alpha}-histone genes is modulated by enhancer and chromatin insulator,” Nucleic Acids Res., Dec;37(22), pp. 7407-7415, 2009.
19. V. Cavalieri, R. Melfi, G. Spinelli, “The Compass-like locus, exclusive to the Ambulacrarians, encodes a chromatin insulator binding protein in the sea urchin embryo,” PLoS Genet., 9(9):e1003847, 2013.
20. G. La Rocca, B. King, B. Shui, X. Li, M. Zhang, K. M. Akat, et al, “Inducible and reversible inhibition of miRNA-mediated gene repression in vivo,” Elife, Aug 31;10:e70948, 2021.
21. V. Cavalieri, M. D. Bernardo, G. Spinelli, “Regulatory sequences driving expression of the sea urchin Otp homeobox gene in oral ectoderm cells,” Gene Expr. Patterns, Jan;7(1-2), pp. 124-130, 2007.
22. V. Cavalieri, M. Di Bernardo, L. Anello, G. Spinelli, “cis-Regulatory sequences driving the expression of the Hbox12 homeobox-containing gene in the presumptive aboral ectoderm territory of the Paracentrotus lividus sea urchin embryo,” Dev. Biol., Sep 15;321(2), pp. 455-469, 2008.
23. V. Cavalieri, G. Spinelli, “Ectopic hbox12 Expression Evoked by Histone Deacetylase Inhibition Disrupts Axial Specification of the Sea Urchin Embryo,” PLoS One, Nov 30;10(11):e0143860, 2015.