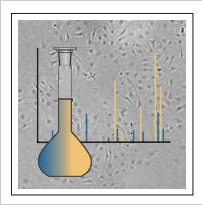
doi:10.2533/chimia.2024.558 Chimia 78 (2024) 558



Highlights of Analytical Sciences in Switzerland

Division of Analytical Sciences

A Division of the Swiss Chemical Society

Exploring Zebrafish Embryonic Cell Line PAC2 by Proteomics Profiling

Mihai-Ovidiu Degeratu[a,b], René Schönenberger[a], Nikolai Huwa[a], and Ksenia Groh[a]*

*Correspondence: Dr. K. Groh, E-mail: ksenia.groh@eawag.ch
[a]Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, CH-8600 Dübendorf; [b]ETH Zurich, Department of Environmental Systems Science, Universitätstrasse 16, CH-8092 Zurich

Keywords: Animal testing alternative · Bottom-up global proteomics · Cell line-based models · Cell morphology markers · Fish toxicity · Zebrafish

Over 350'000 synthetic chemicals are currently used in commerce and this number continues to increase. As many chemicals end up contaminating the aquatic environment, concerns over their effects on the organisms living in water are growing as well. Fish toxicity tests provide crucial data for chemical risk assessments, which in turn are necessary to guide environmental protection efforts. However, these tests sacrifice millions of fish and require ample resources, raising both the ethical and cost-related concerns.

Alternative methods, such as fish-derived permanent cell lines, represent promising animal-free toxicity test models, but their properties need to be better understood to enable broader uptake in research and regulation. For this, molecular profiling could allow insights into both the general characteristics and the functional capacity of these cell lines.

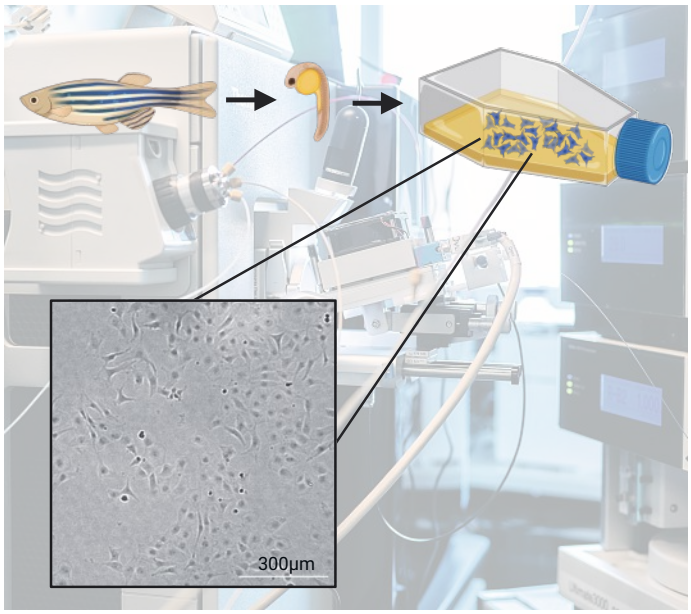


Fig. 1. Permanent fish cell line PAC2 was derived from early embryos of zebrafish (*Danio rerio*) in the 1990s and originally classified as fibroblast-like. Phase contrast image shows recent culture of PAC2 cells from our lab, displaying heterogeneous appearance and notable presence of cells with epithelial-like morphology.

Here, we focused on the PAC2 cell line derived from early embryos of zebrafish (*Danio rerio*). PAC2 was originally classified as 'fibroblast-like' based on cell morphology, but currently, PAC2 cultures in ours and others' laboratories exhibit a heterogeneous appearance with pronounced epithelial-like features instead. Therefore, we set out to validate the initial classification by examining the presence of protein markers characteristic of different cellular origins. For this, we relied on a mass spectrometry-based bottom-up global proteomics approach, an analytical method that not only overcomes the absence of specific antibodies for some of the fish proteins, but also enables simultaneous detection and quantification of multiple proteins without prior knowledge.

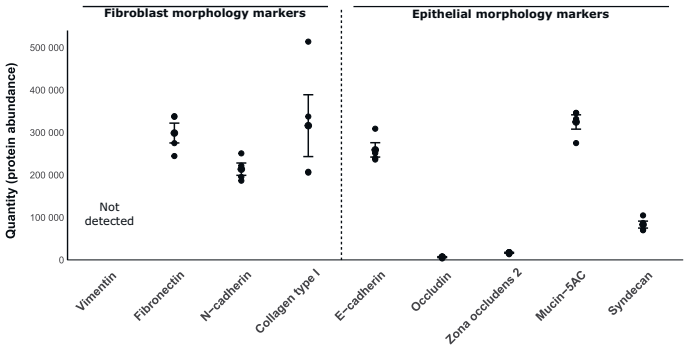


Fig. 2. Protein markers associated with fibroblast and epithelial morphology, measured in actively growing PAC2 cells by bottom-up global proteomics, acquired with nanoLC-MS/MS on Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer in data-independent acquisition (DIA) mode. Each dot represents a biological replicate.

Among the *ca.* 7000 proteins covered by our method, we could observe fibronectin, N-cadherin, and collagen type I, known as fibroblast-associated proteins, but not the most specific fibroblast marker, vimentin. We also detected multiple proteins related to epithelial-like morphology, including E-cadherin, syndecan, mucin-5AC, and tight junction proteins, occludin and zona occludens 2. Our evidence thus suggests that PAC2 cell line harbors mixed cell populations, which questions its official classification as 'fibroblast' only. **This work demonstrates the power of mass spectrometry-based global proteomics analysis for studying non-mammalian cell models.**

Acknowledgement

This research is funded by the Swiss National Science Foundation through National Research Programme 79 'Advancing 3R – Animals, research and society', project number 407940_206439, 'Expanding the fish invitrome towards a modular, socio-technical framework for animal-free prediction of chemical toxicity to fish'.

Received: May 22, 2024

Can you show us your analytical highlight?
Please contact: Dr. Veronika R. Meyer, Unterstrasse 58, CH-9000 St. Gallen
Tel.: +41 71 222 16 81, E-mail: analytical_highlights@chimia.ch