Proteomics profiling questions morphological classification of zebrafish cell line

Mihai-Ovidiu Degeratu, René Schönenberger, Nikolai Huwa, Ksenia Groh

Eawag, ETH domain, Ueberlandstrasse 133, 8600 Dübendorf, Switzerland
Mihaiovidiu.degeratu@eawag.ch

Chemical testing for aquatic risk assessment currently requires high numbers of fish and ample resources, which raises ethical and economical concerns. Cell lines derived from fish tissues/organs represent promising alternative (animal-free) test models for predicting chemical toxicity to fish, but their regulatory uptake has been slow. One hurdle has been the insufficient knowledge about the properties of these cell lines, which could be enhanced by looking at molecular profiles (e.g. proteome). This could help improve our understanding of the cell lines` general characteristics (e.g. cell morphology) as well as their (potential) functional capacity.

Here I present the case of the zebrafish (*Danio rerio*) embryonic cell line, PAC2, which was originally classified as a fibroblast cell line, based on visual inspection. However, this view has been challenged, as PAC2 cells also resemble epithelial-like morphology, especially when they are in a confluent monolayer. To investigate this, I performed mass spectrometry-based bottom-up global proteomics analysis of the PAC2 cell line. Cells were sampled at three cell culture growth phases: early (lag), exponential and stationary. Proteins were digested with trypsin and analysed by nano liquid chromatography (nanoLC) and tandem mass spectrometry (MS/MS) on the Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer (Thermo Scientific), operated in a data-independent acquisition (DIA) mode. Direct DIA analysis was performed with Spectronaut® 18 (Biognosys AG). Further data analysis was done in RStudio.

Our method allowed measuring ca. 7000 proteins in the PAC2 cells. The protein abundance profiles were clearly distinct between the three growth phases. Notably, multiple epithelial cell markers were identified in addition to proteins characteristic of fibroblasts. This calls into question the morphological classification of PAC2 as a purely “fibroblast” cell line. Ongoing work focuses on comparative analyses of proteins and pathways expressed in the three growth phases, seeking to elucidate further the general characteristics and to deduce the (potential) functional capacities of the PAC2 cell line.

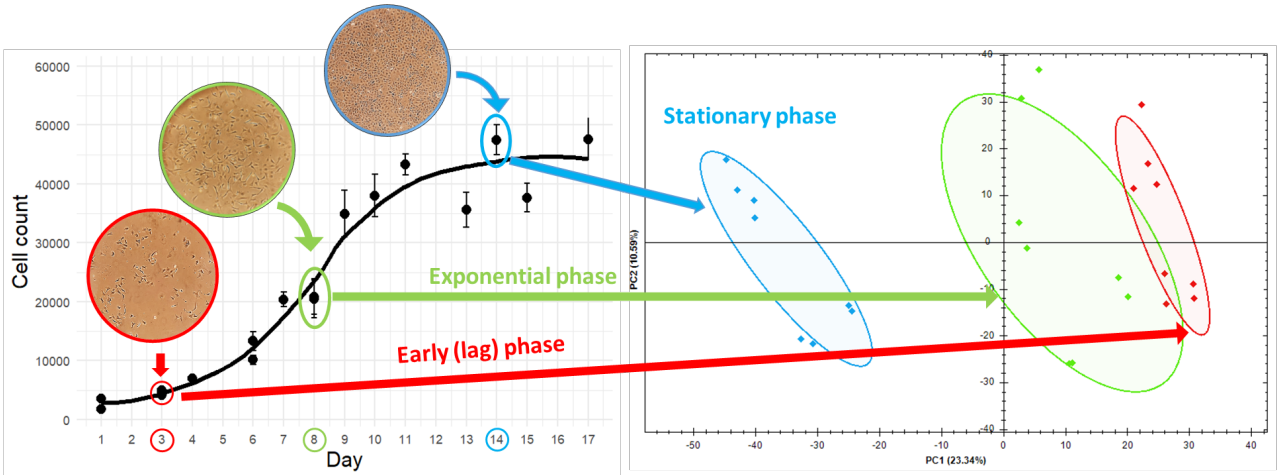

Figure. **Proteomics profiling of PAC2, a fish cell line derived from zebrafish embryos**. Fish cells collected at the early (lag; day 3 post seeding), exponential (day 6 post seeding) and stationary (day 15 post seeding) phases of the cell culture growth display distinct protein abundance profiles, as revealed by the PCA analysis.