CRISPR/Cas9 makes its way into ecotoxicology – A tale of estrogenic antagonism

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Driver?

Endocrine disruptors are exogenous and undesired chemicals that have been in the spotlight for some time now. Their modulating action on endocrine signalling pathways makes them a particularly interesting topic of research within the field of ecotoxicology. Traditionally, endocrine disrupting properties are studied by observing the effects of exposure to suspected chemicals. However, the advent of targeted gene editing tools to directly assess the function of specific genes has been a breakthrough in biology, allowing us to understand molecular mechanisms and to contribute to the development of AOPs. Among these, the CRISPR/Cas9 method has accelerated progress across many disciplines in biology and quickly become a vital tool for the assessment of genetic conditions. This versatile tool affords a plethora of opportunities to different disciplines to assess effects of chemicals. In this case, we applied the tool to assess antagonism by directly inactivating the receptors targeted by endocrine disruptors.

We focused on the developmental effects caused by antagonizing estrogenic signalling using a novel approach by 'knocking out' genes expressing three of the four known oestrogen receptors by means of CRISPR/Cas9. We knocked out the *esr1*, *esr2b* and *gper* genes in zebrafish and performed a panel of biological tests on zebrafish (*Danio rerio*) larvae to determine the effects that selective antagonism may cause.

What the data whispered

1. Craniofacial development

The *esr1* mutants had significant craniofacial deformities, including malformations in structures such as Meckel's cartilage and ceratohyals. This highlights *esr1* as critical in craniofacial cartilage formation. 70% of Esr1 mutants had severe malformations (figure 1).

2. Bone mineralisation and growth

gper1 mutants exhibited reduced bone mineralization in the opercle and shorter standard length at 10dpf, indicating a role of GPER1 in bone development and growth regulation (figure 2a and 2b).

3. Behavioural changes

Both *esr1* and *gper1* mutants showed altered swimming behavior, with decreased activity during light phases, suggesting a role for these estrogen receptors in regulating normal behavioral patterns (figure 3).

Figure 1

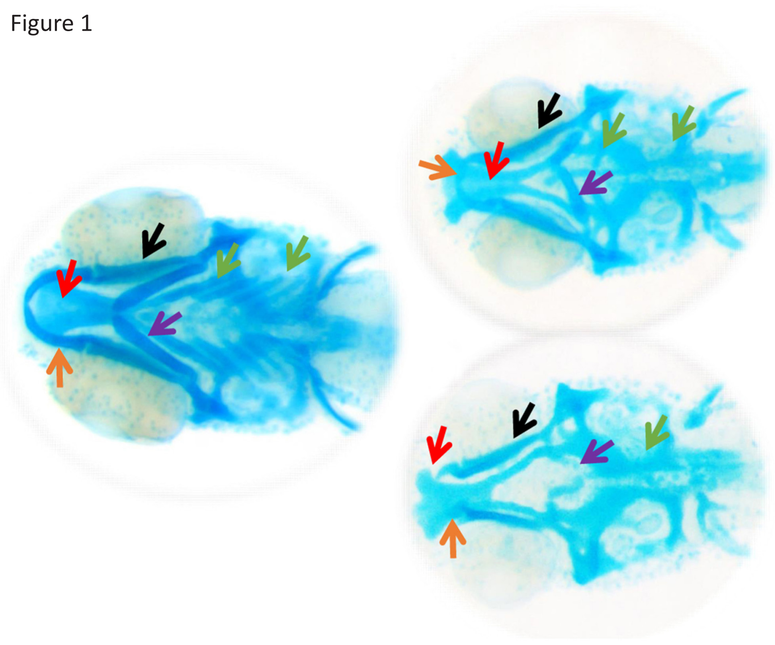


Figure 2a

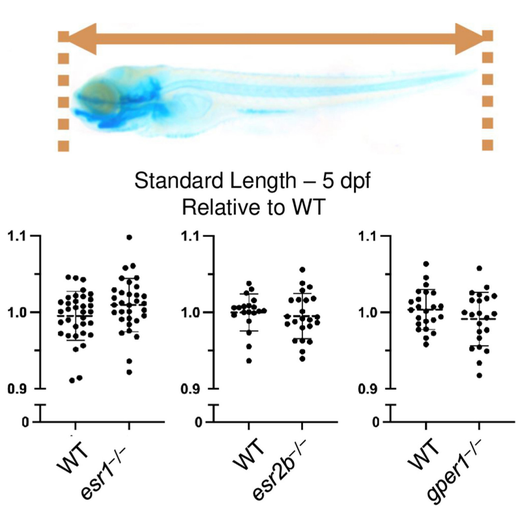


Figure 2b

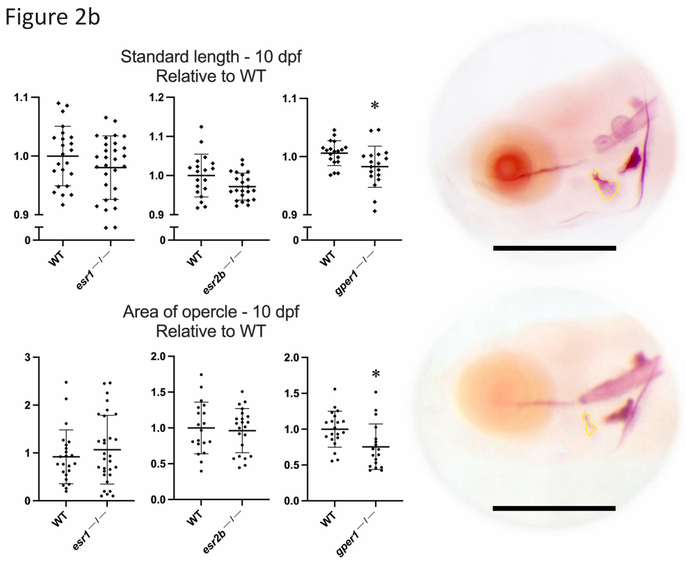
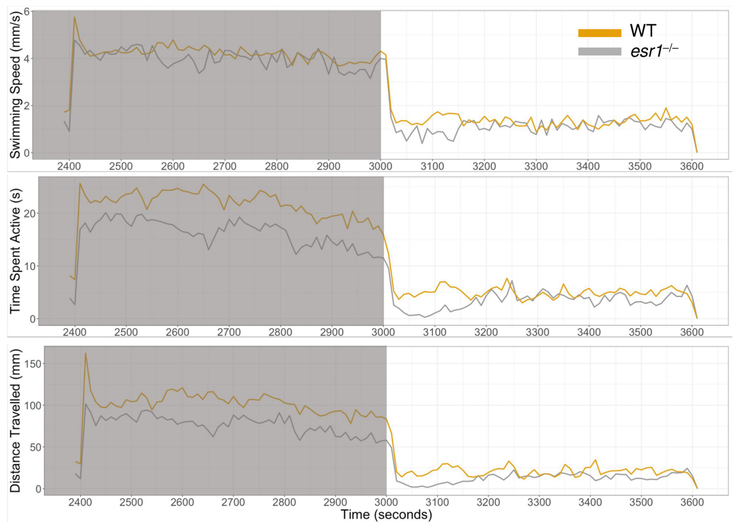


Figure 3



Pocket wisdom and opportunities

- Oestrogen receptors play an important role in early development
- Absence or deficiency of any of the ERs impacted to a certain extent any of the apical endpoints assessed within the biological tests
- No lethality was observed, fish were grossly normal
- Test estrogenic compounds in the absence of ERs

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