LETTER

Reply to Krens et al: Cell stretching may initiate cell differentiation

In their letter, Krens et al. (1) question whether our tissue surface tension model can be applied to zebrafish aggregates and explants. They argue that the stretched-out surface cells shown in our manuscript (2) are differentiated squamous epithelial enveloping-layer (EVL) cells, which implies that these zebrafish explants consist of two tissue types and that our model does not apply to these explants. Their data neither contradict our results nor support their claim that "the model as such has very little predictive value for zebrafish explants" (1).

The authors (1) present time series data for zebrafish aggregates and explants using a transgenic zebrafish line that expresses keratin-EGFP specifically in EVL cells. Their data show that surface cells of zebrafish ectoderm aggregates begin to express keratin-EGFP 4–5 h after incubation (figure 1 a and e in ref. 1) but not at earlier time points. Because we showed aggregates fixed 1–2 h after preparation, the authors' (1) results are consistent with those presented in our manuscript (2). They then cite two studies (3, 4) to support their claim that they were unable to reproduce the absence of EVL markers or observe positional exchange between surface and bulk cells in zebrafish ectoderm aggregates; however, these references neither study surface cell migration in cell aggregates nor conflict with the in situ data that we presented.

We cannot comment on why Krens et al. (1) were unable to reproduce our supplemental movie (Movie S1) (2) that shows that surface and bulk cells exchange positions, because they do not say how they performed this experiment. The exchange of bulk and surface cells might escape observation unless many time-lapse images were captured at several focal planes; however, it certainly happens, because we have previously shown (5) that zebrafish ectoderm aggregates within the time frame of interest exhibit liquid-like behavior including fusion, on contact, into a larger sphere. Here, surface cells must become bulk cells, because the sum of the surface areas of the two original spheres exceeds that of the larger sphere.

Krens et al. (1) also show the expression of tight junction and apical membrane markers as evidence that surface cells differentiate into epithelial cells. We interpret these data differently. As we state in ref. 2, our model requires that "in stretched cells cadherin molecules must diffuse to the (much larger) contact interface," and in *SI Text*, equation S25 in ref. 2, we explain that our model accounts for actin remodeling at contacting interfaces, although this polarization is reversible when surface cells become bulk cells. Because our model requires polarization of surface cells in homogeneous cell aggregates, the data by Krens et al. (1) showing cell polarization at short time points and simultaneous absence of EVL markers are in complete agreement with the model.

Therefore, our model applies to zebrafish ectoderm aggregates before 4–5 h in culture, precisely within the time frame of the experiments performed in our original manuscript. An interesting possibility that deserves further study is that the mechanical deformation could induce the EVL cell fate. A possible connection between increased stress and restriction of EVL division planes that ultimately leads to lineage restriction has been suggested previously (3).

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The authors declare no conflict of interest.

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