RUNX3 is expressed in the epithelium of the gastrointestinal tract

The article by Levanon et al (2011) reports that expression of the transcription factor Runx3 is undetectable in gastrointestinal (GIT) epithelium. These results contradict our results, particularly those in Li et al (2002), which indicate that Runx3 is strongly expressed and functions as a tumour suppressor in GIT. Here, I aim to clarify the issues by summarizing the findings associated with this controversy.

Li et al (2002) (Fig 1I) indicated that mouse Runx3 RNA is expressed primarily in the lower part of the gastric gland where pepsinogen-producing chief cells reside (reproduced here in Fig 1A). Subsequently, we demonstrated the presence of RUNX3 protein in chief cells of human gastric epithelium by immunohistochemistry (IHC; Fig 1B), which was further validated by in situ hybridization (Ito et al, 2005; Fig 1C). Overall, remarkably similar RUNX3 RNA and protein expression patterns in human and mouse gastric epithelium were obtained by three different individuals, 3 years apart. In contrast to these observations, Levanon et al (2011) claim that RUNX3 is absent from mouse GIT epithelium. To document their claim in human tissues, they illustrate this negative result in a single figure (see Supporting Information Fig 2 in Levanon et al, 2011). In addition, Levanon et al (2011) did not discuss Ito et al (2005), which is an unfortunate omission given the relevance of this work to the current discussion.

We show here that Runx3 RNA expression can be detected by RT-PCR in samples from mouse GIT epithelium. We have previously noted that the level of cellular Runx3 RNA is low (Ito et al, 2009). Strikingly, exon 4 and LacZ are absent from a large fraction of Runx3 RNA in GIT epithelium of Kyoto-Runx3 KO and exon 3 is directly linked to exon 5 (Fig 2). The Kyoto-Runx3 KO we sent to Israel in 2010 have been continuously bred for about 10 years, which may have resulted in exon 4 skipping and explain why Levanon et al could not observe LacZ staining in GIT epithelium of Kyoto-Runx3 KO (Levanon et al, 2011). Evidence of strong Runx3 expression in mouse GIT that we observed around 1998–2002 and the induction of Runx3 upon genotoxic stress will be discussed in detail elsewhere.

In their paper, Levanon et al first use Runx3\textsuperscript{LacZ/−} mice (Rehovot-Runx3 KO) to conclude that Runx3 is not expressed in mouse GIT. This mouse strain was reported in 2002 (Levanon et al, 2002) and their properties of Rehovot-Runx3 KO were later described as follows:

(1) This genetic modification did not result in a Runx3-null allele since the p33 Runx3 splice variant escaped the knockout strategy (Supporting Information Fig S1, upper left panel; Fainaru et al, 2004).
(2) The p33 protein is highly active and required for dendritic cell maturation (Puig-Kroger et al, 2010).
(3) p33 transcripts do not contain the LacZ ORF (Levanon et al, 2003; Levanon and Groner, 2009) since the LacZ cassette was inserted in exon 2 (Levanon et al, 2002).
(4) p33 transcripts are a minor transcript in normal mouse but, in Rehovot-Runx3 KO, it constitutes the major transcript in bone marrow-derived dendritic cells (Supporting Information Fig S1, upper right panel; Fainaru et al, 2004).
(5) This results in lack of LacZ staining in the affected tissues.

In 2001, Levanon/Groner traced the expression of Runx3 through LacZ staining in the Rehovot-Runx3 KO (Levanon et al, 2001). Since the p33 transcript does not have LacZ, one would surmise that LacZ staining cannot be observed in bone marrow-derived dendritic cells of Rehovot-Runx3 KO. Indeed, the lack of LacZ expression in the Runx3-positive epidermal cells in Rehovot-Runx3 KO was reported (third paragraph on p. 1481, Raveh et al, 2005).

It is thus unclear whether Rehovot-Runx3 KO can be used to accurately trace Runx3 expression through the use of LacZ expression. Accordingly, the Rehovot-Runx3 KO results in Mech Dev, 2001, indicating lack of Runx3 expression in epithelial cells, should be re-evaluated. Since they continued using Rehovot-Runx3 KO (Fig 2; Levanon et al, 2011), Levanon/Groner should examine whether splicing of Runx3 mRNA is

![Figure 1. Assortment of figures from earlier publications demonstrates the expression of Runx3/RUNX3 in mouse and human stomach epithelial cells.](image-url)
skewed in epithelial cells. Yet, there is no mention of the p33 transcript in Levanon et al (2011).

Levanon/Groner subsequently generated new mouse strains to reach the same conclusion as that obtained by Rehovot-Runx3 KO. Inconsistent with their conclusion is the flow cytometry data (Fig 5G, Levanon et al, 2011), which suggests Runx3 expression in EpCAM+ cells in adult intestine. There is a low but definite peak at intensities ranging higher than 10^4, thus indicating the presence of Tomato fluorescence in EpCAM+ fraction. The data suggest the existence of a small population of epithelial cells expressing high Runx3 levels. Furthermore, at the range of 10^5–10^6 intensity, there may be a significant number of Tomato-positive cells in EpCAM+ fraction. With these hints for Runx3 presence in epithelial cells, Levanon et al should re-examine why the other methods they employed failed to detect Runx3 in the GIT epithelium. Since Levanon et al (2011) employ T cells as positive control in the intestine during IHC, their approach would have missed the much lower Runx3 expression in epithelial cells.

Furthermore, they used embryonic tissues to examine Runx3 expression in stomach and adult tissues to examine the intestine.

Levanon/Groner devoted considerable effort to generate new mouse strains instead of focusing on human tissues. The significant amount of data on mouse embryos presented in Levanon et al (2011) is only remotely relevant to human cancer.

In summary, I stress that the conclusions of Levanon et al (2011) are not well supported because critical information, be it references or relevant KO mouse characteristics, was omitted. Moreover, they did not follow up on clues that could have led to positive detection of Runx3 in the GIT epithelium. As such, they should re-evaluate their conclusion.

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