Diverse Tumor Pathology due to Distinctive Patterns of JAK/STAT Pathway Activation Caused by Different Drosophila polyhomeotic Alleles

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ABSTRACT Drosophila polyhomeotic (ph) is one of the important polycomb group genes that is linked to human cancer. In the mosaic eye imaginal discs, while phdel, a null allele, causes only non-autonomous overgrowth, ph505, a hypomorphic allele, causes both autonomous and non-autonomous overgrowth. These allele-specific phenotypes stem from the different sensitivities of ph mutant cells to the Upd homologs that they secrete.

We have previously reported that different ph alleles cause tissue overgrowth in different ways. Although a ph null allele, phdel, causes only non-autonomous cell overproliferation, a ph hypomorphic allele, ph505, causes both autonomous and non-autonomous cell overproliferation (Feng et al. 2011) (Figure 1, A–C). In mosaic tissues, we define overproliferation of mutant cells as autonomous and overproliferation of genotypically wild-type cells induced by mutant cells as non-autonomous. We have also elucidated the signaling pathway involved in phdel-induced non-autonomous cell overproliferation. In summary, elevated Notch activity in ph cells up-regulates the expression of JAK/STAT pathway ligand Upd homologs, which in turn activate the JAK/STAT pathway in neighboring wild-type cells and cause their overproliferation (Feng et al. 2011). In this study, we addressed why both a ph null allele and a ph hypomorphic allele cause tumors but in such different ways.

We first tested whether the same signaling pathway underlay non-autonomous overproliferation induced by both phdel and ph505. The functions of Notch and Upd homologs in the ph505 mosaic eyes were examined with the same strategy used for phdel (Feng et al. 2011). A ph505-Notch double-mutant line was generated, and eyes mosaic for this line were essentially of the same size as wild-type eyes (Figure 1, D vs. F). The mosaic eye discs had normal size and normal cell proliferation level, as shown by PH3 staining, which marks mitotic cells (Figure 1, H vs. J). Moreover, the size of ph505-Notch clones was significantly reduced when compared to that of ph505 clones (Figure 1, I vs. J). These results indicated that Notch was required for both autonomous and non-autonomous overproliferation induced by ph505.

We next recombined ph505 with upd3, a deficiency line that lacks all three upd homologs in the Drosophila genome (Feng et al. 2011). Mosaic analyses were then performed using this double-mutant line. ph505-upd3 mosaic eyes were significantly smaller than ph505 mosaic eyes and were comparable to wild-type eyes (Figure 1, D, E, and G), indicating that tissue overgrowth was largely suppressed. PH3 staining of the double-mutant mosaic eye discs showed that these discs had relatively normal size and cell proliferation level (Figure 1, H vs. K). Importantly, ph505-upd3 clones were also drastically reduced in size compared to ph505 clones (Figure 1, I vs. K). These results indicated that Upd
homologs are required not only for non-autonomous but also for autonomous cell overproliferations induced by \( \text{ph}^{505} \).

It is not surprising that the same signaling pathway is responsible for non-autonomous overproliferation induced by both \( \text{ph}^{\text{del}} \) and \( \text{ph}^{505} \), and it is not completely unexpected that Notch is also required for \( \text{ph}^{505} \)-induced autonomous overproliferation, as Notch is a transcription factor that has been shown to autonomously regulate cell proliferation (Artavanis-Tsakonas and Muskavitch 2010). However, the three Upd proteins are secreted ligands (Hombria et al. 2005; Arbouzova and Zeidler 2006) and are not expected to have any direct effect on autonomous cell proliferation.

To interpret our observations, we hypothesized that \( \text{ph}^{505} \) cells still respond to Upd ligands secreted by themselves in an autocrine or paracrine manner and therefore overproliferate. On the other hand, \( \text{ph}^{\text{del}} \) cells were no longer responsive to Upd ligands.

To functionally test this hypothesis, we again applied the double-mutant strategy, taking advantage of the fact that the genes \( \text{domeless} \) [\( \text{dome} \), encoding the only transmembrane receptor of the \( \text{Drosophila} \) JAK/STAT pathway (Brown et al. 2001)] and \( \text{hopschotch} \) [\( \text{hop} \), encoding the only \( \text{Drosophila} \) JAK kinase (Binari and Perrimon 1994)] are also on the X chromosome as is \( \text{ph} \). We first recombined \( \text{ph}^{505} \) with two \( \text{dome} \) alleles to generate \( \text{ph}^{505} \text{-dome} \) double-mutant lines. Eye discs mosaic for these lines were still significantly larger than wild type, but the size of double-mutant clones was dramatically reduced, so that only a tiny portion of the disc was composed of mutant cells (Figure 2, A and B). PH3 staining indicated that the non-autonomous proliferation level was still high, but autonomous proliferation largely disappeared (Figure 2, A and B). We further examined the adult eyes mosaic for such double-mutant lines and found that these eyes were still much larger than wild type and similar to \( \text{ph}^{505} \) mosaic eyes in size, but they generally were not folded as seen in \( \text{ph}^{505} \) mosaic eyes (Figure 2, H, J, and K).

Next we generated a \( \text{ph}^{505} \text{-hop} \) double-mutant line. We found that autonomous proliferation in mosaic eye discs of this double mutant was also significantly suppressed, with mutant cells accounting for only a small portion of the whole disc. On the other hand, non-autonomous cell overproliferation was not affected, and the overall size of these discs was still significantly larger than wild type (Figure 2C).

Adult eyes mosaic for this double mutant showed similar phenotypes as those of \( \text{ph}^{505} \text{-dome} \) mosaic eyes. These eyes were still significantly larger than wild type, but they were generally not folded (Figure 2L). Therefore, the removal of either \( \text{dome} \) or \( \text{hop} \) from \( \text{ph}^{505} \) cells only suppressed autonomous overproliferation and did not affect non-autonomous overproliferation, making such double-mutant mosaic discs phenotypically similar to \( \text{ph}^{\text{del}} \) mosaic discs.

As controls, \( \text{ph}^{\text{del}} \text{-dome} \) and \( \text{ph}^{\text{del}} \text{-hop} \) double-mutant lines were also generated using the same \( \text{dome} \) and \( \text{hop} \) alleles. Mosaic analyses on eye discs showed that the removal of
dome or hop from phdel cells did not affect non-autonomous overproliferation. It did, however, mildly reduce the mutant clone size (Figure 2, D–F), suggesting that phdel cells might still have a weak response to Upd ligands. Adult eyes mosaic for these double-mutant lines were phenotypically indistinguishable from phdel mosaic eyes (Figure 2, I and M–O), consistent with the above observations in mosaic eye discs.

Finally, we asked why phdel and ph505 cells respond differently to the Upd ligands secreted by themselves. We hypothesized that some of the JAK/STAT pathway modulators might be differentially expressed in phdel and ph505 cells. To test this hypothesis, we chose TU-tagging, a technique that enables the purification of RNA from mutant cells without having to physically isolate such cells (Miller et al. 2009).

Brieﬂy, Drosophila is unable to synthesize uridine monophosphate from uracil due to the lack of phosphoribosyltransferase (UPRT). When exogenous UPRT is expressed in mutant cells by MARCM (Lee and Luo 1999), such cells acquire the ability to utilize uracil. If these larvae are fed with 4-thiouracil (4-TU), a uracil derivative that contains a thio
group, only mutant cells would be able to use 4-TU and eventually incorporate thio-containing uridine into newly synthesized RNA. This treatment has little toxicity, and the thio-labeled RNA can be purified from total RNA using conventional biochemical methods.

We performed TU-tagging to isolate RNA from ph\textsubscript{del} cells and ph\textsubscript{505} cells and used quantitative RT-PCR to examine candidate gene expression (Figure 3A). The expression of the JAK/STAT pathway receptor dome was significantly higher in ph\textsubscript{505} cells than in ph\textsubscript{del} cells. A higher receptor expression might sensitize ph\textsubscript{505} cells to the Upd ligands. The levels of enok and socs\textsubscript{42a}, both negative regulators of the JAK/STAT pathway (Arbouzova and Zeidler 2006; Müller \textit{et al.} 2008), were also significantly higher in ph\textsubscript{505} cells compared to ph\textsubscript{del} cells. This might represent feedback loops that negatively regulate the pathway activity. In fact, several such negative feedback loops, in which elevated pathway activity upregulates a negative pathway regulator, have been reported in JAK/STAT pathway (Arbouzova and Zeidler 2006).

Together, we conclude that both ph\textsubscript{del} and ph\textsubscript{505} cause autonomous overexpression of Upd homologs in mutant cells, which represents the only driving force of cell overproliferation in ph\textsubscript{del} and ph\textsubscript{505} mosaic tissues and in essence acts non-autonomously to activate JAK/STAT pathway. The different phenotypes of these two types of mosaics are due to different sensitivity of mutant cells to Upd homologs. ph\textsubscript{505} mutant cells robustly respond to Upd ligands that they secrete. Therefore, Upd ligands secreted by ph\textsubscript{505} cells simultaneously induce overproliferation in both mutant and wild-type cells. In contrast, ph\textsubscript{del} cells are largely insensitive to Upd ligands, so that Upd ligands secreted by ph\textsubscript{del} cells induce only overproliferation in wild-type but not mutant cells. Furthermore, differential expression of the JAK/STAT pathway receptor dome might underlie the different sensitivity of ph\textsubscript{del} and ph\textsubscript{505} cells to Upd ligands. Models of cell proliferation patterns and the underlying signaling pathways in ph\textsubscript{del} and ph\textsubscript{505} mosaic tissues are given in Figure 3, B and C.

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**Literature Cited**


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