Chapter 2

New Insights from Drosophila into the Regulation of EGFR Signaling

Nicholas Harden

Abstract

Genetic analysis of Egfr signaling in Drosophila has a long-standing track record of making important contributions to our understanding of the Egfr pathway. While the central Ras/MAPK pathway has long been well defined, there is much to learn with regard to its cross talk with other pathways and how it is regulated. A better understanding of the regulation of Egfr signaling is of particular interest with regard to the participation of misregulated Egfr signaling in tumorigenesis. Recent studies in the fly have led to some surprising results, identifying regulators of Egfr acting in unexpected ways.

Key words EGFR, *Drosophila*, Signaling, Ras/MAPK, Tumorigenesis

1 Introduction

Genetic analysis in Drosophila has made tremendous contributions to our understanding of Egfr signaling and its regulation. In particular, the fly has been very informative in revealing details of the temporal and spatial regulation of Egfr signaling, including providing important insights into processing of the Egfr ligand Spitz and transcriptional induction of positive- and negative-feedback on the pathways [1, 2]. Positive regulators turned on by the pathway include the ligand Vein and Rhomboid, a protease that cleaves Spitz, whereas negative regulators include Argos, which forms nonfunctional heterodimers with Spitz, Kekkon, which forms heterodimers with Egfr, and Sprouty, an inhibitor of the Ras/MAPK pathway. Egfr signaling regulates the development of multiple tissues throughout the life cycle of the fly, including the eye and wing. Recent work in the latter two tissues has identified four new regulators of Egfr signaling, including the surprising findings that two well-known oncogenes antagonize the pathway, and thus Egfr signaling may have a tumor suppressor function (Fig. 1) [3–6].

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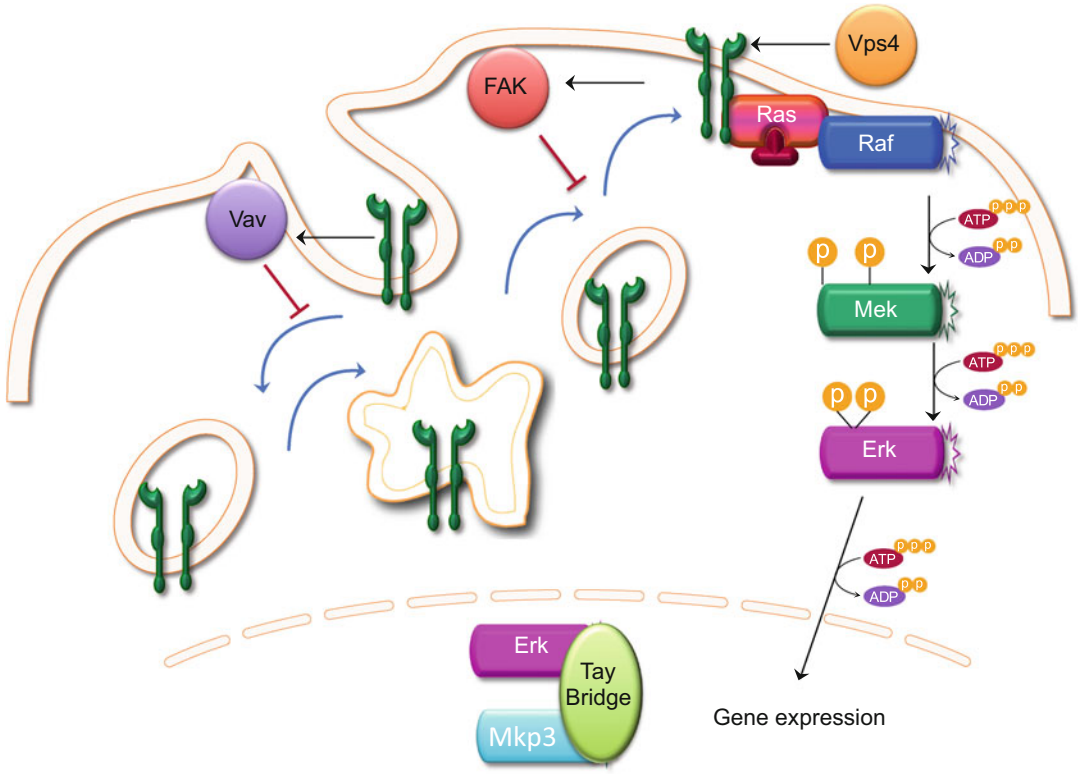


Fig. 1 Recent studies in Drosophila have identified four new regulators of Egfr signaling, with three of these, FAK, Vav, and Vps, likely acting upstream of pathway activation. FAK might inhibit Egfr signaling by repressing recycling of the receptor, whereas Vav might block endocytosis required for maximal Egfr signaling. Vps4 might be acting independently of protein trafficking at the receptor level, whereas Tay Bridge appears to negatively regulate Egfr signaling at the other end of the pathway in the nucleus

2 FAK

Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase that interacts with integrins in focal adhesions and has been implicated in tumor cell survival and invasion [7]. FAK interacts with and is phosphorylated by several receptor tyrosine kinases including RET. This interaction was further investigated in Drosophila by Macagno and colleagues, who made the unexpected finding that FAK operates in a negative feedback loop, being activated by excessive RET activity but then inhibiting RET-driven phenotypes [4]. The authors went on to extend their study to FAK regulation of Egfr signaling, and the findings were similar: Egfr induced FAK activation and co-overexpression of FAK with Egfr in the eye suppressed the effects of excessive Egfr signaling. FAK's ability to inhibit signaling by two RTKs appears to be through impairment of the Ras/MAPK pathway, and this FAK function is conserved in a breast cancer cell line MDA-MB-231 [4]. Furthermore, in these cells FAK

inhibits Egfr recycling to the membrane, which could be a mechanism for impairment of Egfr signaling. These various results indicate that FAK might act as a tumor suppressor in cells with elevated RTK activity, and suggest that caution should be observed in the use of FAK inhibitors in cancer therapies.

3 Vav

Vav is a guanine nucleotide exchange factor for the Rho subfamily small GTPases expressed in many cancers, which has been shown to be activated by Egfr [8]. Martin-Bermudo et al. recently studied the interaction of Vav with Egfr signaling using the Drosophila eye, and made the surprising discovery that rather than functioning as a oncogene and positive effector downstream of Egfr, Vav inhibits Egfr signaling [3]. Thus, as with FAK, Drosophila genetics has revealed potential tumor suppressor function for a well-known oncogene. *vav* mutant pupal retinas had increased cell numbers including extra photoreceptors. Several readouts for the Egfr pathway showed that consistent with the increased cell proliferation, Egfr signaling was elevated in *vav* mutant ommatidia. Strikingly, halving the dose of the Egfr ligand Spitz in *vav* mutants substantially rescued the eye defect, confirming that Vav antagonizes Egfr signaling in the eye. By creating new *vav* alleles using CRISPR/Cas9, the authors were able to show that the inhibitory function of Vav required both the GEF activity and binding to Egfr.

As with FAK, a possible area for Egfr regulation by Vav could be in protein trafficking, which has context-specific effects on Egfr signaling and might explain the unexpected finding that two well-established oncogenes could act as tumor suppressors with regard to Egfr signaling in a particular tissue. The regulation of Egfr signaling by endocytosis is complex and Egfr internalization can make both positive and negative contributions to Egfr signaling [9]. In Hela cells, Vav2 expression delays Egfr endocytosis, whereas knockdown of Vav2 enhances Egfr degradation and inhibits cell proliferation. In these cells, Vav has an oncogenic role, but in a tissue where endocytosis is required for maximal Egfr signaling Vav could function as a tumor suppressor. Consistent with this, work on the vesicle protein Myopic indicates that endocytosis is required for efficient Egfr signaling in the Drosophila eye [10].

4 Vsp4

Thus far, we have seen two oncogenes acting more like tumor suppressors with regard to Egfr signaling in Drosophila, possibly through effects on Egfr trafficking. The surprises continue with the recent finding that a known participant in endocytosis as part of the

ESCRT-III complex, Vps4, positively regulates Egfr signaling in an endocytosis-independent manner [6]. In studies performed in the Drosophila eye and wind discs, Legent showed that clones of *Vps4* mutant cells showed reduced expression of Egfr target genes despite having increased levels of Egfr protein. Epistasis analysis positioned Vps4 at the level of Egfr activation, with expression of the processed version of the Spitz ligand unable to rescue Egfr-dependent photoreceptor differentiation in *Vps4* mutant cells whereas activated Ras could. The effect of loss of Vps4 on Egfr signaling was opposite to that of mutations in other ESCRT-III complex genes, which prompted Legent et al. to determine if Vps4 was functioning in endocytosis in its regulation of Egfr signaling [6, 11]. The authors addressed this by performing epistasis analysis in the eye using clones of cells double mutant for *Vps4* and *shibire* (*shi*), which encodes dynamin, a GTPase that internalizes Egfr, and is a negative regulator of Egfr signaling [12, 13]. The increased Egfr signaling in *shi* mutant eye tissue causes excessive photoreceptor differentiation, but this is blocked when cells are additionally mutant for Vps4, suggesting that Vps4 is required for Egfr activation upstream of endocytosis. Further studies on the effects of Vps4 on Egfr signaling in the ovary were consistent with a model in which Vps4 favors Egfr signaling in an endocytosis-independent manner. It remains to be determined how Vps4 contributes to Egfr signaling; it does not appear to be at the level of transcription or translation of the receptor; one possibility proposed by Legent et al. is that it could be acting indirectly on Egfr at the cell surface through effects on lipid raft composition.

5 Tay Bridge

For our last new regulator of Egfr signaling, we move from the cell surface to the other end of the pathway in the nucleus. Tay Bridge (Tay) is a nuclear protein that was picked up in a gain-of-function screen for genes that when overexpressed cause effects on wing vein differentiation [5]. Overexpression of Tay caused the elimination of longitudinal veins, a phenotype similar to loss of Egfr signaling. Conversely, loss of Tay resulted in excess wing vein differentiation similar to excessive Egfr signaling. Genetic interaction studies were consistent with Tay functioning as a negative regulator of Egfr signaling, for example loss of Tay enhanced the extra vein formation caused by expression of RasV12 or ectopic Rhomboid. Furthermore, Tay overexpression enhanced the loss-of –vein phenotype caused by reduction of Egfr signaling. To determine if Tay was directly regulating the Egfr pathway, Molnar and de Celis looked at several readouts for the pathway: the accumulation of di-Phosphorylated Erk (dP-Erk) and expression of Egfr target genes. Consistent with Tay functioning as a negative regulator of the pathway, these readouts were reduced with Tay overexpression and increased with loss of Tay.

At what level is Tay regulating the Egfr pathway? Tay is a nuclear protein and the authors focused on nuclear events in Egfr signaling. Following its production by Egfr activation, dP-Erk moves into the nucleus where it regulates gene expression by phosphorylating transcription factors. The phosphatase Mkp3 dephosphorylates and inactivates dP-Erk in the nucleus, and the authors found that Tay binds both Erk and Mkp3, suggesting that it might negatively regulate Egfr signaling by sequestering Erk in the nucleus.

These four recent papers on Egfr signaling in the fly have revealed some unexpected findings about the regulation of this pathway that will be of interest to address in vertebrates. Of particular note are the findings that two well-known oncogenes, *fak* and *vav* are behaving as tumor suppressors with regard to Drosophila Egfr, suggesting that their functions with regard to oncogenic pathways may be context-dependent. Furthermore, proteins similar to Tay may not always negatively regulate Egfr signaling. Expression in the Drosophila wing disc of human AUTS2, which has a 250-amino acid stretch of homology to Tay, causes activation of Egfr signaling [5]. These various results add to a growing list of findings that many genes implicated in cancer exhibit an "antagonistic duality" with regard to the regulation of tumorigenesis, and indicate that they may not be good therapeutic targets [14].

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