IDENTIFICATION OF NEW GENES INVOLVED IN TUMUROGENESIS

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INTRODUCTION

The Ras family of GTPases function to transduce signals from receptor tyrosine kinases that promote cell survival and proliferation. Activation of Ras signaling occurs in ~ 30% of human cancers. However, activated Ras alone is insufficient to produce malignancy. Thus, the discovery of genes cooperating with Ras in cancer is imperative to understand tumor growth driven by Ras activating mutations.

This context has been successfully used to isolate genes that when mutated would enhance the tumorigenic capacity of Ras^{V12}. One of them is PVRAP, which was isolated as a possible regulator of oncogenic Ras. Additionally, several putative mutants PVRAP were identified . In this work, we have tested the capacity of PVRAP to enhancer oncogenic Ras and have analyze at the molecular level the isolate putative mutants.

To do this, the *Drosophila Melanogaster* imaginal wing disc, which is of epithelial origin, is used as a model.

OBJECTIVE

The objective of the present project is to analyse the role of PVRAP as regulator of oncogenic Ras^{V12}

METHODOLOGY

The function of the gene PVRAP was knocked down using two approaches:

1) Used the systema UAS-GAL4 for system and the interference RNA (RNAi) technique
2) Generation of a collection of Drosophila PVRAP mutants generated by the CRISPR technique

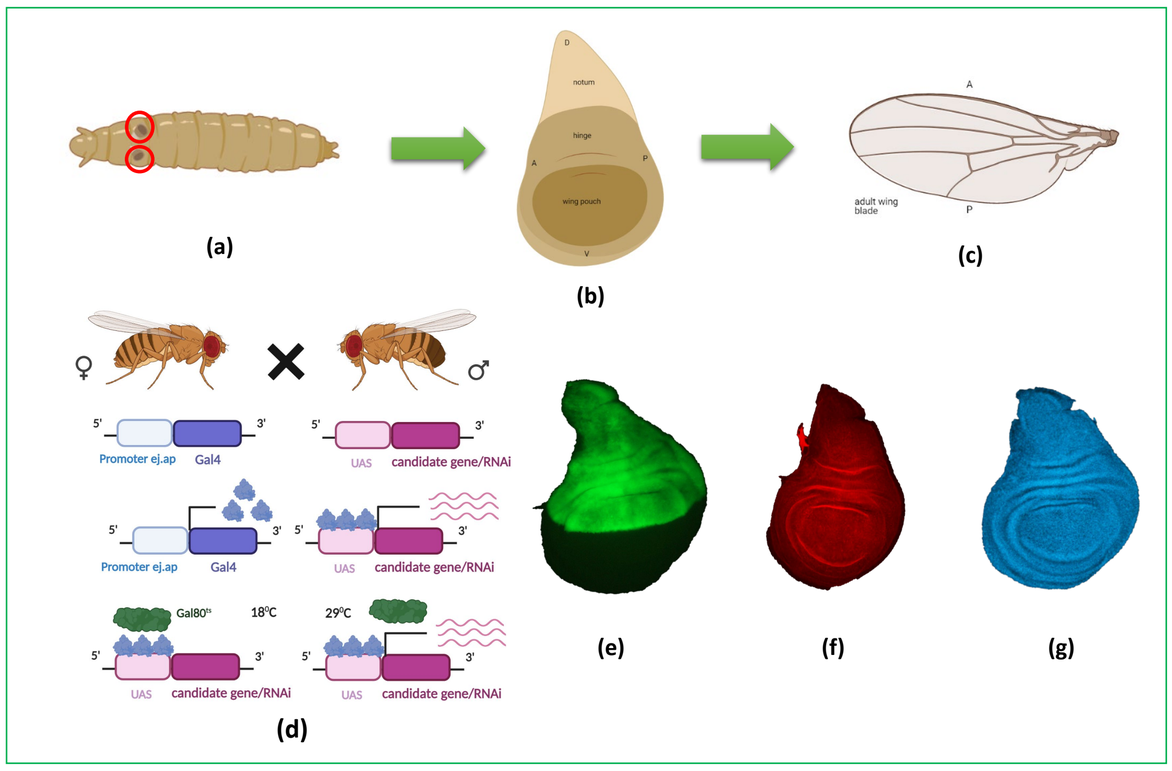

Figure 1. **(a)** Third-instar larvae. Wing discs are enclosed by red circles. **(b)** Scheme of a Drosophila wing disc. D, A, P and V respectively refer to dorsal, anterior, posterior and ventral. **(c)** Structures derived from the wing disc: the wing proper and the hinge. **(d)** Schematic representation of the GAL4/UAS system for the expresión of a gene of interest from a tissue-specific driver. **(e)** A wing disc with the genotype ApGal4; UAS-GFP(Ap>GFP) stained with antibody against GFP (green). **(f)** A wing disc with Rhodamine Phalloidin to detect F-actin (RhPh, red). **(g)** The nuclear marker Hoechst (DNA, blue). Stock images from Biorender.com

RESULTS

1. Role of PVRAP as regulator of oncogenic Ras^{V12}

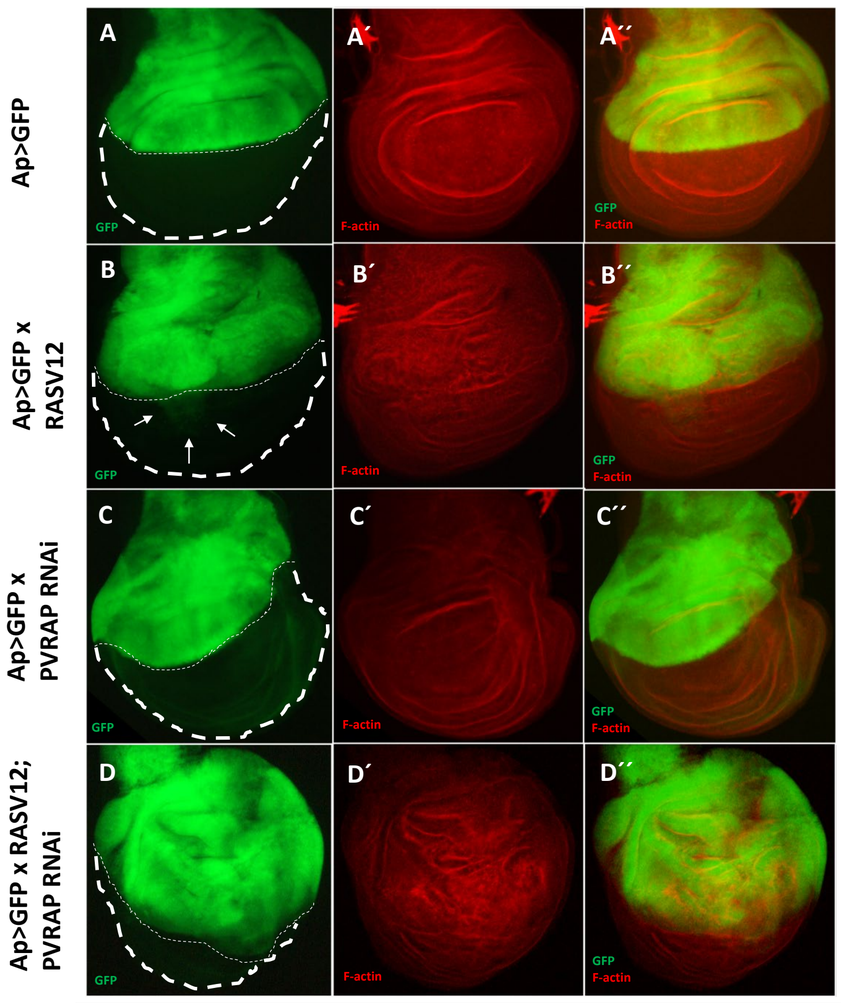

Figure 2. Wing discs from third-instar larvae stained with antibody against GFP (green) and Rhodamine Phalloidin to detect F-actin (RhPh, red) to label cell suffers. Lines were added for improved visualization: dotted lines delimit the apterous domain; dashed lines outline the wing disc shape. Arrows point to different groups of tumor cells that migrated out of the expression region of the ap driver gene. **(A)** Wing disc expressing GFP in the dorsal domain using the apterous Gal4 line (Ap>GFP). **(B)** Co-expression of Ras induces ectopic folds. In Ap <GFP Ras^{V12} wing discs, several groups of GFP-stained cells escaped the apterous domain, but the shape and limits of this domain is maintained. **(C)** Co-expression of PVRAP RNAi in a wild type does not increase the Ras phenotype. **(D)** Co-expression of PVRAP RNAi + Ras^{V12} increases Ras^{V12} phenotype. In Ap> GFP x Ras^{V12}; PVRAP RNAi wing disc, the apterous domain greatly exceeds its original area, invading other areas of the wing discs. In addition, groups of cells also observed out of the Ap domain.

2. Generation of isolate putative mutants

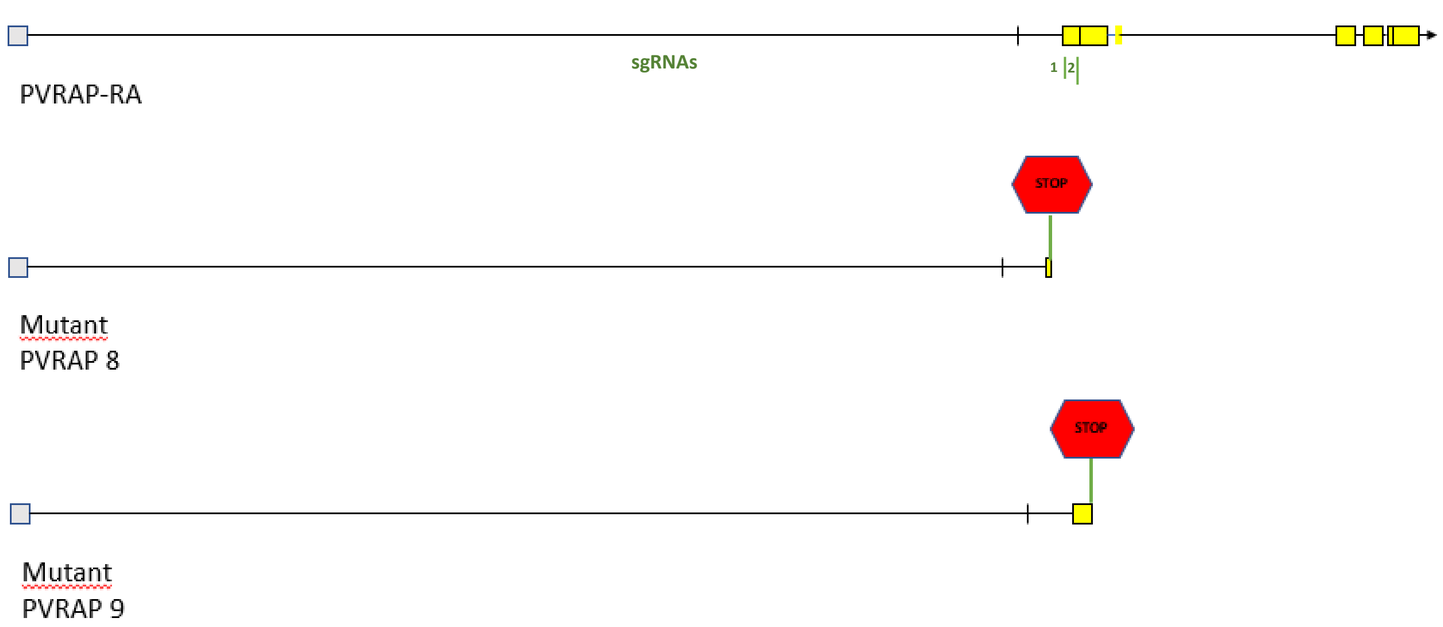

Figure 3. Two gRNAs were designed to respectively target the first exon of PVRAP. In both mutants there has been a change in reading pattern.

3. Generation of PVRAP mutant alleles supports its role as modulator of Ras ^{V12} -mediated tissue hyperplasia

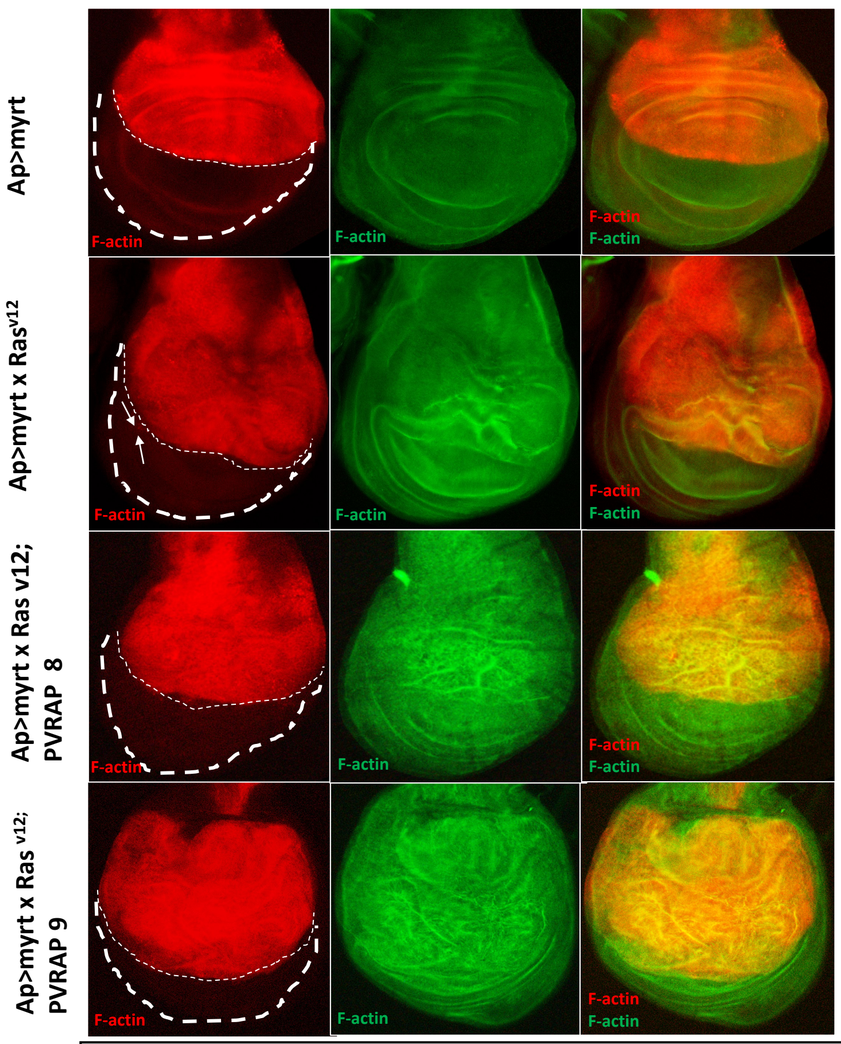

Figure 4. Images of wing imaginal discs from third-instar larvae stained with Rhodamine Phalloidin to detect F-actin red and green. Co-expression of PVRAP 9 + Ras^{V12} increases Ras^{V12} phenotype more than co-expression with PVRAP 8. On the other hand, a greater number of ectopic folds is observed in PVRAP 9.

4. The apterous domain area is greater in PVRAP 9

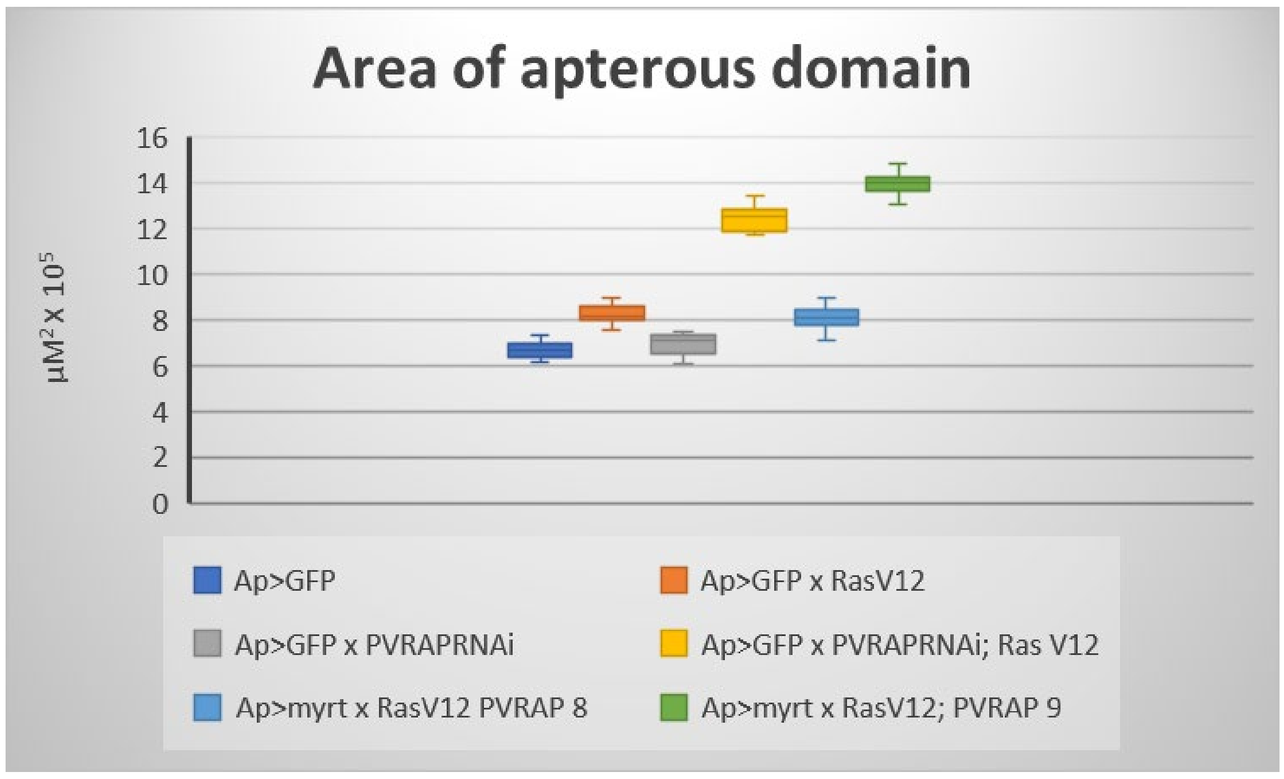

Figure 5. This graph shows that PVRAP 9 has a greater apterous domain stained area than PVRAP 8, the opposite of what was expected, since PVRAP 8 should express a stronger phenotype. Therefore, another putative mutant isolate will be studied later with a mutation similar to PVRAP 8. In this graph the population is N= 20.

CONCLUSION

PVRAP as a enhancer of Ras^{V12} tissue hyperplasia