Generation of a novel *D. melanogaster* platform to elucidate oncogenic activity of common human p53 missense mutants

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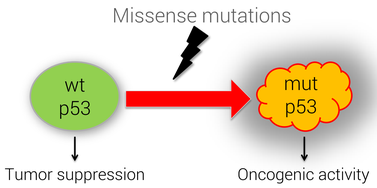
Introduction

The tumor suppressor p53 prevents uncontrolled cell growth by three separate mechanisms: inducing apoptosis, initiating cell-cycle arrest, and activating DNA repair mechanisms in response to cell damage. Due to its central role in tumor eradication, it is unsurprising that p53 mutations are found in over half of human cancers. Unlike all other tumor suppressors however, 75% of these are missense mutations, with just six of them accounting for a third of all mutations found in the DNA binding domain of p53. Recent findings indicate that mutations in these "hotspot" locations may encode gain of function oncogenic activity to p53. Given their high prevalence, these mutations suggest a previously underappreciated selective advantage. Here, we use D. melanogaster as a novel in vivo platform to explore human p53 oncogenicity of these missense mutations.



Rationale & Materials and Methods

The broad rationale is the placement of a human p53 construct into flies with the regulatory framework maintained. Humanized wild type and mutant flies will then be examine for phenotypic (germ cell activation and co-localization to the egg chamber nuclei) and functional (apoptosis in embryos) differences. Irradiation is used to induce p53 expression.

Studying Drosophila human p53 (hp53) mutants offers us several advantages:
-DNA binding domain and p53 regulatory network are conserved
-lack of p63, p73, and mdm2 prevent redundancy and confounding effects
-hp53 has been shown to be able to bind to and activate the dmp53 response elements in vivo

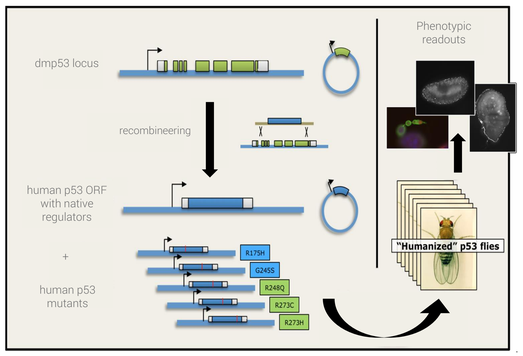


Figure 1. The human p53 wt or mutant genes were inserted into the background of a dmp53-/- fly to study the effects of wild type hp53 *in vivo*.

To study these mutations, we first profiled the expression patterns of wild type and mutant hp53 in the fly and their ability to rescue dmp53 function. Expression levels of p53 were determined by immunofluorescence, while biological function was determined by the use of a GFP biosensor that specifically reports dmp53 activity and vital dye acridine orange staining to identify dying cells in irradiated embryos.

Results

Germ cell activation (immunofluorescence assay)

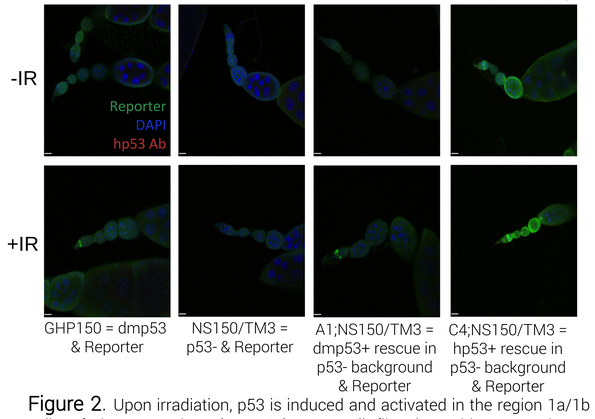


Figure 2. Upon irradiation, p53 is induced and activated in the region 1a/1b stem cells of the germarium (GHP150). p53 null flies lose this expression pattern (NS150/TM3), but are rescued by a dmp53 rescue insert (A1; NS150/TM3). It is also shown that our human p53 rescue (C4;NS150/TM3) reverts to the wild type dmp53 phenotype as well, though with a much stronger signal.

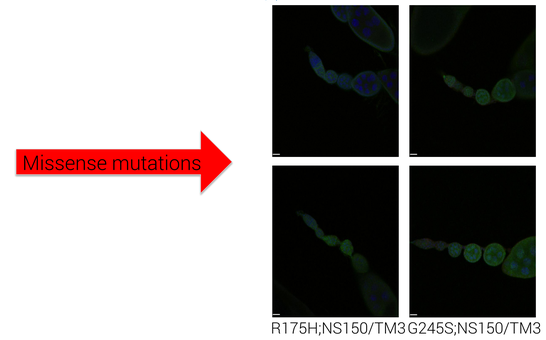
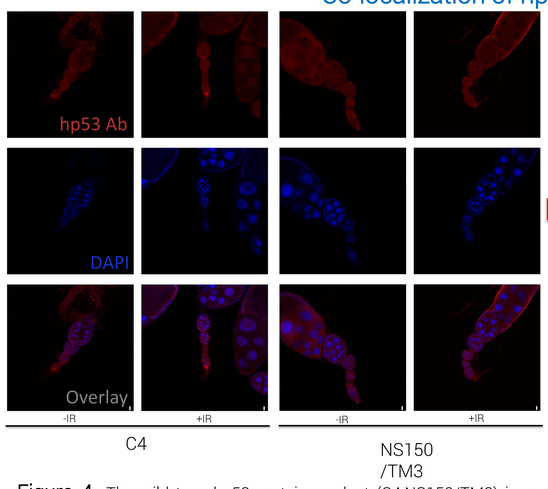


Figure 3. Both mutants shown (R175H & G245S) lack IR-induced expression of p53 in the region 1a/1b stem cell as in the hp53 wild type. Other mutants recapitulate this phenotype.

Co-localization of hp53 to the egg chamber nuclei



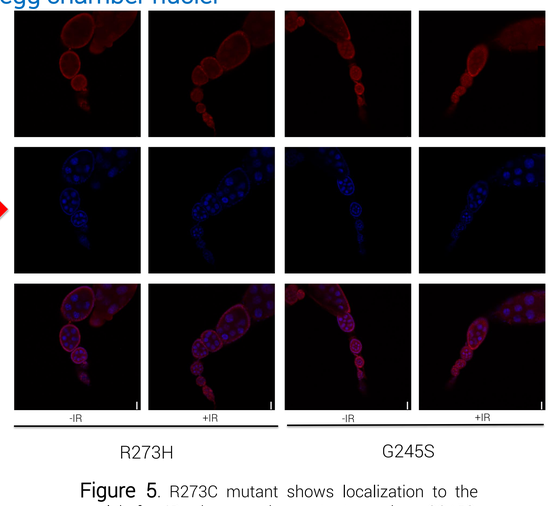
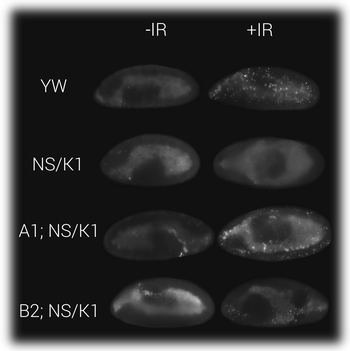


Figure 4. The wild type hp53 protein product (C4;NS150/TM3) is capable of localizing to the nucleus of the egg chamber both with and without irradiation. Oddly, the p53 null germarium (NS150/TM3) exhibits an exclusion from the egg chamber nuclei regardless of irradiated state. dmp53 localization cannot be determined as no antibody has yet been manufactured against its protein product.

Figure 5. R273C mutant shows localization to the nuclei after IR, whereas other mutants, such as G245S, show localization both with and without IR. Additionally, R248Q (not shown) do not exhibit localization.

Apoptosis in embryos (functional assay)

Figure 6. Apoptosis is examined by the vital dye acridine orange, a DNA chelator that stains patches of cells bright white in the images shown. Functional evidence is demonstrated by the reproduction of the WT phenotype (YW) by both the dmp53 rescue (A1;NS150/TM3) and hp53 rescue (C4;NS150/TM3) when compared to the p53 null fly in both irradiated embryos and wing discs.



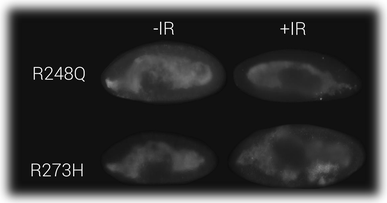


Figure 7. Mutants R248Q and R273H are shown as lacking apoptotic mechanisms. This is only accurate for ~50% of mutants so far analyzed, with others demonstrating the appropriate IR-induced phenotype.

Conclusions

We first demonstrate that both functional activity and expression of dmp53 are rescued *in vivo*, demonstrating we have successfully created a platform with which to study human p53 missense mutations. Furthermore, phenotypic expression and functional activity differences of mutants are discussed below.

Germ cell activation
- Our endogenous reporter is activated in stem cells (region 1), but absent in p53 null. This phenotype is rescued with a dmp53 insert.
- The wild type hp53 is highly activated but localized to the proper stem cell region.
- Mutants activate reporter less well and do not exhibit strong activation in stem cells

Egg Chamber Co-localization
- Human p53 can co-localize to most nuclei, except in the p53 null and R273H, where it appears to be excluded from nuclei
- Other mutants show a range of phenotypes, from localization without irradiation to absolutely no localization to nuclei

Apoptosis in embryos
- YW embryos acquire cell death after IR, whereas p53 null flies do not
- The wild type hp53 rescues the YW phenotype
- Mutants do not exhibit IR-induced apoptosis, in most cases

Future Direction

-Finish all mutant lines
-Finish functional assays of wing discs
-Quantitate expression levels of hp53 compared to dmp53 in our platform.
-Determine precise localization of foci/puncta within the germarium and their significance.
-Determine oncogenicity of more complex cancer models (invasion/metastases).

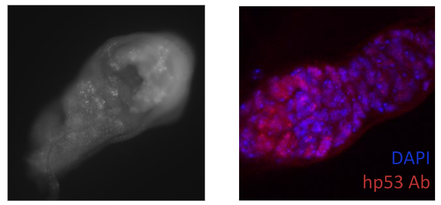


Figure 8. Wing disc showing acridine orange stain (left). Foci seen in germarium with hp53 antibody of hp53 rescue line (right.)

Acknowledgements

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