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A genetic screen for new mutations affecting the signaling pathway by which cells respond to TGF-β-related factors in Drosophila was carried out. The genetic background was sensitized using a mutation in the Type I receptor gene. New mutations affecting the phenotypes caused by the sensitized genetic backgrounds were recovered. The new mutations were mapped to six different genes. Three of these genes were previously shown to participate in the signaling pathway, confirming that the sensitized genetic background was appropriate. DNA sequencing is being used to confirm the generation of new mutant alleles in the Mad gene and the Type II receptor gene. Three of the genes in which new mutations were recovered map to regions not previously known to be involved in dpp signaling. Detailed genetic mapping has provided cloning strategies for the molecular identification of two of these genes.

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J. Michael Huff 10/28/96

PI - Signature Date

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INTRODUCTION:

The TGF-β growth and differentiation factors have been implicated in the regulation of breast epithelial cell proliferation and in the invasive behavior of metastatic breast cancer (for example: Pierce et al., 1995). The goal of our research is to identify the molecules involved in the signal transduction cascades activated by TGF-β. Defects in the type II TGF-β receptor are associated with different human tumors (for example: Markowitz, S. et al., 1995). Recently the Mad proteins have been implicated as proteins on the TGF-β signaling pathway (Zhang et al., 1996). Mutations in the Mad proteins Madr2 and Dpc4 have been reported in colon and pancreatic carcinomas (Eppert et al., 1996; Hahn et al.,1996). We predict that other, currently unknown, molecules in the signaling cascades may also be affected during tumor progression.

In order to apply a genetic approach to the dissection of this signaling pathway, we have proposed to use the TGF-β-related genes and related receptor genes in the fruit fly Drosophila. Because of the high degree of evolutionary conservation, we predict that the Drosophila genes identified in our screens will provide the necessary molecular probes for the identification of the homologous genes in the human genome. This working hypothesis has been confirmed by work on Mad, a gene identified by genetic methods in Drosophila (Sekelsky et al., 1995) that was used to identify human homologs Madr1- Madr4 (Zhang et al., 1996).

RESULTS:

We characterized mutations in the Drosophila gene thickveins, which encodes a type I receptor essential for signaling by the TGF-β-related Drosophila ligand, dpp. Over the past year we have determined that a mutation in thick veins gene (tkv6) sensitizes the genetic background such that mutations in other known genes of the dpp signaling pathway cause detectable phenotypes. In this genetic background, recessive loss-of-function mutations in the Drosophila type II receptor punt or in the Mad gene have a dominant phenotypic effect. We concluded that in the tkv6 genetic background, other genes on the pathway become dosage-sensitive such that a reduction in the wildtype copy number from two to one causes a detectable phenotype. In this case, the phenotypes detected

include changes on the adult cuticle such as loss of distal structures from the legs and defects in the fusion of the dorsal notum.

Over the past year we screened 10,000 mutagenized chromosomes in an F2 screen for second site modifier mutations that enhanced the tkv6 phenotype. We recovered and placed into stock 14 mutations. These mutations have been mapped by several criteria. They were tested for their ability to complement mutations in known genes on the pathway. Importantly, five of the mutations failed to complement mutations in Mad, two failed to complement mutations in punt and one failed to complement mutations in Medea. The recovery of new alleles in three genes known to be on the pathway confirmed the appropriateness of the genetic background and the screening procedures. We are currently sequencing the new alleles of Mad and punt to provide molecular characterization of these alleles.

The other six mutations are being mapped by meiotic recombination and complementation tests with known deletions. They identify three different complementation groups with one, one and four alleles in each. Because these mutations were generated with the chemical mutagen ethylnitrosourea which causes single base changes, we have screened for gamma-ray induced recessive lethals in each of the three complementation groups. These F2 lethal screens successfully identified new alleles in two of the complementation groups for which we had only single alleles. Gamma rays were used in an effort to produce chromosome rearrangements that would help in locating the position of the gene and provide a lesion detectable on Southern blots during the cloning strategies. We are currently in the process of trying to clone two of these three new loci.

CONCLUSIONS:

We have successfully carried out a medium scale mutagenesis for genetic modifiers in the signaling pathway of the TGF-beta-related dpp protein in Drosophila. Recovery of new alleles of Mad and the type II punt receptor indicate the screen was successful. Mutations identifying three other, potentially novel genes have been recovered and characterized. Molecular cloning strategies for two of these genes have been identified and are in progress. This is consistent with the goals and time table initially proposed. We are

also continuing our genetic screens in order to generate additional modifier mutations.

In summary, our progress thus far is on track with the Statement of Work (Appendix G, pg. 23) of our original proposal. We have made good progress in the first 24 months on Task #1 and #2 and are beginning Task #3 on schedule.

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