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DISSERTATION SUMMARY

The functional investigation of the interaction between p53 and *Drosophila melanogaster* TAF(II)155/Bip2 and the human homolog, TAF3

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The tumor suppressor gene *p53* plays a pivotal role in safeguarding the integrity of the genome (Levine. 1997). Most human tumors have a mutation in the *p53* gene or a functional defect in the p53 pathway, highlighting its importance for preventing tumorigenesis. p53 is a sequence-specific transcription factor. Normally, the amount of p53 protein in a cell is kept at a low level. Cellular stresses, such as DNA damage, hypoxia, or abnormal oncogene activation, signal to p53, stabilize and activates it as a transcription factor, p53 in turn arrests cell cycle, induces repair and apoptosis. The mechanisms activating p53 are not fully characterize therefore we were interested in identifying novel proteins that regulate p53. The discovery of *Drosophila melanogaster* p53 (Dmp53) facilitated the examination of p53.

Using yeast-two-hybrid screen several new interacting partners of Dmp53 were identified. One of the identified genes- TAF(II)155/Bip2- was chosen for further analysis. Bip2 is a novel *Drosophila* TATA-box Protein Associated Factor (TAFII), is also named TAF(II)155/Bip2 and its human homologue is a TAF3 (Pointud et al. 2001). The TFIID, RNA polimerase II transcription factor is composed of TATA-binding protein (TBP) and TBP-associated factors (TAFs). The TAF(II)155/Bip2 and its human homologue TAF3 protein contain a Histone Fold Domain (HFD) in the N-terminal region and a Plant Homeodomain (PHD) in the C-terminal region (Gangloff et al. 2001).

Because of the results received in yeast-two hybrid experiment we examined the possible interaction between the human homologue of these *Drosophila* proteins. Surprisingly, the *Drosophila* homologue (Bip2) can also interact hp53 and with the family members of hp53 (hp73α and β) not only with Dmp53 in a yeast-two hybrid assay. First we amplified the hTAF3 from HeLa cDNA library, but we could not amplify the full length form of hTAF3, only two partial length forms. In our experiments we used this longer and shorter forms. To examine if there is any effect of the overexpression of the human homologue (TAF3) on the transactivation activity of hp53, and the members of hp53 family (p73α, p73β), we transfected HeLa cells with the hp53 and TAF3 overexpressing constructions and a luciferase reporter plasmid, we found that the overexpressing of TAF3 decreased the transactivation activity of hp53 and p73β and a lesser extent in the case of p73α. Using RNA interference (RNAi), we investigated the effect of the elimination of TAF3 protein on the transactivation activity of hp53. We created double-stranded RNAs to the TAF3 and we cotransfected it with hp53 expressing plasmid and a luciferase reporter plasmid into HeLa cells. In some cases the dsRNA-TAF3 was able to decrease the transcriptional activity of hp53, but in other cases we could not detect this result. To examine the subcellular localization of TAF3 we transfected HeLa cells with TAF3 overexpressing vectors and we stained the cells with primary and fluorescein-conjugated secondary antibody, we found that the hTAF3 protein showed really nuclear localization in HeLa cells. To further prove the physical interaction in vitro between the interacting partners and Dmp53, we used GST-pull down assay, we found that the TAF(II)155/Bip2 is able to bind to the Dmp53. The other method that we used is the Immunoprecipitation (IP), but by this technique we could not detect interaction between hp53 and the two different size of TAF3. On the other hand it is possible that the TAF3 cDNA clone in our hand does not contain the region recquired for interaction. Therefore we will use the full length mouse homologue of TAF3 (TAFII140), which was amplified and offered for us by our cooperating partner.

We plan to investigate the interaction between hp53 and mTAF3 (TAFII140) using of GST-pull down and IP experiments.

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