Ceske Budejovice, 8th June 2010

Review of the bachelor thesis of Eugen Kubala: Study of the Role of Mutations L39Q, L49Q and N78T of Casein Kinase I epsilon in Breast Cancer Using Drosophila Melanogatser as a Model organism.

In his bachelor work Eugen Kubala wanted to characterize the role of particular mutations of casein kinase Iε in cancerogenesis by creating transgenic flies with insertion of the genomic DNA construct coding for the fly CKIε homologue (called *dco*) bearing the same mutations as those found in several cases of human breast cancer. The aim of the work was to prove that mutated form of CKIε may be responsible for the initiation of cancer and that mutations found in patients are indeed functionaly important. This topic is highly interesting and the *Drosophila* model perfectly suited to answer such a question. Eugen tried hard to inject flies with the transgene but he did not succeed in creating any viable flies that would stably integrate it into the genome. However, it is obvious that Eugen understood well the topic, he thought it through and he is aware of the possible causes of his set-back.

The introduction is well and clearly written and methods described in detail but in order to pin-point the cause of embryonic lethality after injection a couple of more controls could have been done:

- After several unsuccessful attempts Eugen could have used a positive control to rule out any technical problem during his injections (perhaps to inject flies with the original unmutated CKIe plasmid or any UAS-construct). He mentions in the discussion that similar controls were done but as I understand these were performed by a different person on different occasions so although suggestive they can not serve as a proper internal controls.
- Why did Eugen choose the particular combination of the three mutations (L39Q, L49Q and N78T) for his experiments? There are 19 mutations found in the samples from human breast cancer patients. Is this the most aggressive form of cancer? And if yes, could a different combination of mutations cause less severe effects and perhaps not cause embryonic lethality after injections? Eugen mentions in the discussion that flies with individual mutation L39Q were made. What was their phenotype?
- What is the nature of the mutations from the patients. Are they gain of function or do they work as dominant negative?
- What is the usual survival rate of embryos after injection with other constructs? It would be good to include this information into the Result table for comparison (or at least mention it in the Discussion).
- Although not very probable it is possible that the transgene was inserted correctly after the injection but the white gene from the plasmid was not expressed. Did Eugen sequence the gene to make sure that no random mutation has been selected?

Eugen's English and terminology is sometimes a little bit awkward but at least it shows that he really wrote the thesis by himself. I suggest excellent mark for his work.

RNDr. Alena Krejci, PhD Alena Krejci