Evaluation of the thesis by MUDr. Petr Libý entitled "Thyroid hormone receptors and selected interacting proteins in glial tumors: The analysis of the expression and regulatory potential."

The thesis is arranged in the standard way and has 79 pages. It includes in addition the list of publications and list of presentations of Dr. Libý. The thesis is written very well, and despite the fact of not being an expert in the field, I could readily understand its content. The documented studies are at the best level of today´s possible approaches, are well conducted and constitute the excellent basis for the presented Ph.D. thesis. I particularly appreciate the originality of the studies!

The presented thesis is based on two experimental articles published in a standard biological journal (Folia Biologica) and an unpublished part dealing directly with the first asked question: the expression and function of thyroid receptors in human tumors of glial or more specifically astrocytic origin. The topic is, in my opinion, extremely difficult to address. It was namely expected from the time of cloning of thyroid receptors in 1the 80ties of the last century that blocking the transcription regulation function by thyroid receptors has the potential to prevent proper cell differentiation and, accordingly, may be a part of the cancer promoting process. Despite the unprecedented accumulation of the knowledge of thyroid hormone receptors and related proteins, our understanding of the biological roles of these molecules is just at its beginning. Apparently, the hormone–transcription activation cascade is only the backbone of the regulation which is further modified and super-regulated by a "myriad" of other factors.

Dr. Libý attempted to characterize the expression of thyroid receptors (TRα1, TRβ1, TRβ2, TRα2) in non-malignant gliosis tissue and in astrocytic tumors using biochemical, molecular biology and immunocytochemical methods: reverse transcription, PCR, quantitative PCR, Western blot and immunocytochemistry. The studies must have been quite difficult for many reasons. As it is stated in the thesis, a very limited mass of the bioptic tissue was available and used under very stringent ethical rules. It is the nature of astrocytic tumors that many of them are necrotic and this may change many factors, all this being naturally subjected to the sampling error. It is also important to mention that despite the effort of many research groups, it is widely assumed that the good antibodies specific for TRα1 are not available. This seems to reflect some basic important characteristic of TRα. Petr Libý accepts this possibility and thinks that proteins recognized by the TRα1 antibodies should be purified and characterized. Good point for him.

The part describing the expression of TRs is complemented by expression studies of RXRs, two selected interacting proteins (SKIP, HDAC3) and Survivin, a protein linked to the regulation of transcription as previously described.

SKIP is a transcription cofactor the function of which is not well understood, it is a cofactor of VDR and probably other NHRs, Notch pathway and likely many other pathways. HDAC3 has a clear connection to TRs since it was shown to be involved in the transcription repression by unliganded TRs and able to interact even with liganded TR

via cyclin D1. The part describing the expression of HDAC3 is well described and characterized. Authors show elevated expression in malignant glial tumors and severe deregulation on the cell level. It became clear only recently, that HDAC3 may be localized in the cytoplasm and the described cellular pattern may reflect the dual, or even multilevel, function of HDAC3.

Surprisingly, the expression of Survivin, known to be linked to proliferating cells and most tumors, was found barely significantly elevated in glial tumors. How can this be explained? Can the glial tissue be actually a "bad" control?

The last part of the thesis focuses on BIR-1 loss-of-function and gain-of-function in one larval stage of C. elegans. Authors did a whole genome screen for the effect of bir-1 loss-of-function and identified genes with decreased expression. Then the authors characterized the expression of selected collagen genes in animals overexpressing bir-1 and confirmed that genes repressed by bir-1 loss-of-function are strongly induced in bir-1 overexpressing animals. This must have been technically a quite demanding study but it complements well the previousely published work from the same laboratory.

What is the novelty in the thesis? The part concerning the expression of TRs in glial tumors is important but the only conclusion that can be made out of it is that TRs are very often expressed more than in control tissues. The minimal conclusion that made by Dr. Libý is that TRs are more likely to be present in glial tumors than decreased or mutated, and it may be anticipated that for such powerfull TFs, they are likely to be used in some pathologic (path)way. Although the collection of cases is restricted due to obvious reasons, the conclusion seems to be valid and important. The fact that such a study was not published twenty years after the discovery of the genes may reflect similar difficulties encountered by other groups. This is indeed supported by the findings of elevated expression of interacting proteins, the dimerizing partners RXRs, especially RXRg, HDAC3, and I agreed with the author, the Survivin. If Survivin really interacts with TRs as suggested, such interaction is potentially very important.

I have a few questions to Dr. Libý:

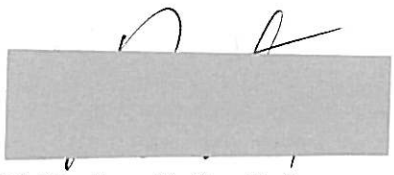
1) What is the pattern recognized by commercial antibodies and antibodies obtained in cooperation with other group on cell and Western blot levels?

2) The short form of HDAC3, the HDAC3D, was detected as strong bands in some tumors while it was completely missing in other tumors. Could a nonspecific interaction with another (blood) protein be ruled out?

3) The function of the short form of HDAC3 was not studied. Did the author attempted to use the HDAC3D in transfection experiments? What is author´s expectation of the biological role of HDAC3D?

4) If BIR-1 augments transcription on genome wide level and in the thyroid receptor containing system, what mechanism is expected to take place in this regulation?

Conclusion: I strongly recommend that, after the successful defense, MUDr. Petr Libý is, according to the valid rules relevant for the postgraduate education, awarded the title Doctor of Philosophy.



Prague, May 17, 2007

Prof. RNDr. Ivan Raška, DrSc.