



In situ observation of DLK1 and DIO3 tumorsuppressor genes in zebrafish



Márta Reining¹, Réka Balogh¹, Erna Balogh¹, Henriett Butz², Kinga Németh², Katalin Bakos¹, Gyöngyi Gazsi¹, Judit Kövesi¹, Andrea Csepeli¹, Róbert Kovács¹, Zsolt Csenki¹, Béla Urbányi¹ and Attila Patócs²

¹Department of Aquaculture, Institute of Environmental and Landscape Management, Faculty of Agricultural and Environmental Sciences, Szent István University, Páter K. Street 1., H-2100 Gödöllő (Hungary)

²HAS-SE Momentum Hereditary Endocrine Tumours Research Group, Szentkirályi Street 46., H-1088 Budapest (Hungary)

Introduction: 15% of intracranial tumors are pituitary adenomas. Pituitary tumors can be either hormone producing or hormonally inactive. Adenomas usually are benign, and their symptoms are mostly related to the pressure exerted by the growing tumor on the surrounding tissues. The exact molecular background of the development of adenomas is not yet clear, however the role of several tumor suppressor genes, oncogenes and miRNAs has been discovered in tumor formation. In humans the expression of genes and several miRNAs mapped to the DLK1-MEG3 locus was found to be decreased in pituitary adenomas compared to healthy pituitary. As the adenohypophysis and hypothalamic-pituitary system (HPS) of zebrafish show great similarity to mammals, zebrafish (*Danio Rerio*) could be a useful model to study the molecular background of pituitary tumor formation.

Methods: The most important 14 human miRNAs and 6 genes were compared to their murine (*Mus musculus*) and zebrafish homologues in silico (Figure 1.). The expression of the *dlk1* (delta-like 1 homolog (*Drosophila*); ID: 101883341, and *dio3* (deiodinase, iodothyronine type IIIb; ID: 798872) was examined by in situ hybridization in zebrafish larvae (5 dpf).

Figure 1. Genes that were investigated in silico

Human	Name	Transcript	Location	Mouse	Transcript	Loation	Zebrafish	Transcript	Location
DLK1	delta-like 1	5	14kr.	Dlk1	10	12kr.	dlk1	1	17kr.
RTL1	retrotransposon-like 1	1	14kr.	Rtl1	1	12kr.	-	-	-
DIO3	deiodinase, iodothyronine, type III	1	14kr.	Dio3	2	12kr.	dio3b	1	20kr.
							dio3a	1	17kr.
MEG3	matrenally expressed 3	27	14kr.	Meg3	11	12kr.	-	-	-
MEG8	matrenally expressed 8	7	14kr.	Meg8_1	1	MG4180	-	-	-
				Rian	17	12kr.	-	-	-
PTTG1	Pituitary tumor-transforming gene	7	5kr.	Pttg1	10	11kr.	pttg1	4	14kr.
PTTG2	Pituitary tumor-transforming gene	1	4kr.	-	-	-	-	-	-
PTTG3p	Pituitary tumor-transforming gene	1	8kr.	-	-	-	-	-	-
PTTG4p	Pituitary tumor-transforming gene	1	14kr.	-	-	-	-	-	-
-	-	-	-	Pttg1ip (interactin g protein)	9	10kr.	pttg1ipb	2	9kr.
-	-	-	-	-	-	-	pttg1ipa	1	6kr.

Results: The examined genes show a high degree of sequence similarity (i.e. Dlk1: 76%; Dio3: 86%; Rtl1: 78%; Pttg1: 81%; Meg3: 79%; Rian: 69%), in mammals while homology is less pronounced in zebrafish (dlk1: 74%; dio3: 71%; pttg1: 91%). miRNAs and some genes from this locus have no homologues in zebrafish.

Expression of DLK 1 gene was detected in the brain and jaw. As DLK1 gene plays a role in embryonic pluripotent neural stem cell differentiation in mammals, they may have the same role in zebrafish (Figure 2.).

Expression of DIO3 gene was also detected in the brain and spinal cord too. DIO3 encodes a functional selenoenzyme that inactivates thyroid hormones (T4 and T3) in mammals. Thyroid hormones mediates the proliferation and differentiation of growing tissues. In accordance with literature data it is expressed in the zebrafish hindbrain, midbrain, forebrain, otic vesicle and pronephric duct. We detected expression in the case of control sense probe too. From the literature we know that in mammals the DIO3 gene is an imprinted gene and has antisense transcript too. This antisense mRNA could be hybridze to the sense probe (Figure 3.).

The in situ hybridization of the above mentioned genes is in progress in adult zebrafish brain sections.



Figure 2. *dlk1* in situ on 5dpf larvae A and D: antisens 55 x magnification B and E: antisens 72 x magnification C and F: sens control 55 x magnification

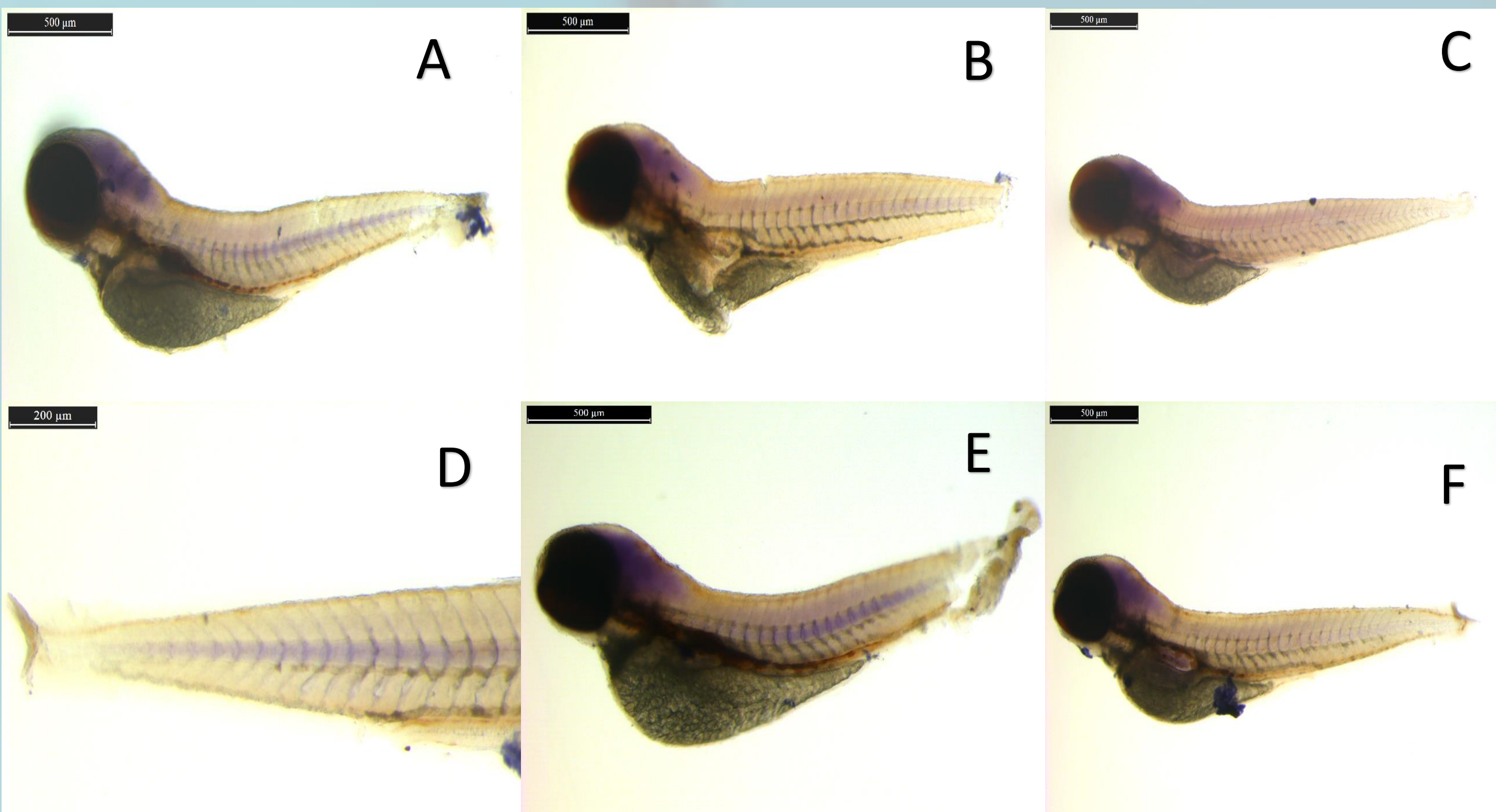


Figure 3. *dio3* in situ on 5dpf larvae A,B and E: antisens 55 x magnification D: antisens 72 x magnification C and F: sens control 55 x magnification



Acknowledgment: The work was supported by the Research Centre of Excellence - 11476-3/2016/FEKUT registry number of applications.

