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Sirotčí jaderný receptor TLX (NR2E1) v regulaci buněčné reprodukce a diferenciace

Orphan Nuclear Receptor TLX (NR2E1) in Regulation of Cell Reproduction and Differentiation

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Contents

Abbreviations

Abbreviation	Meaning
aa	amino acid
ARID1A	AT-rich interactive domain-containing protein 1A
ARs	androgen receptors
C. elegans	Caenorhabditis elegans
CAR	constitutive androstane receptor
CBP	cAMP response element binding protein
CNS	central nervous systém
COUP	chickem ovalbumin upstream promoter
CREB	cAMP response element binding
DAPI	4′,6-Diamidino-2-phenylindole dihydrochloride
DBD	DNA binding domain
ERs	estrogen receptors
FXR	farnesoid X receptor
GRIP1	glucocorticoid Receptor-interacting protein 1
GRs	glucocorticoid receptors
HAT	histone acetyl-transferase aktivity
HNF-4	hepatocyte nuclear factor 4
hTLX	human TLX protein is an orphan nuclear receptor that is expressed in vertebrate forebrains
LBD	ligand binding domain
LXR	liver X receptor
MCM2	minichromosome maintanace protein 2
MRs	mineralocorticoid receptors
NCOA	nuclear Receptor Co-activator
NCoR	nuclear receptor co-repressors
NHR	nuclear hormone receptor
NHRs	nuclear hormone receptors
NR	nuclear receptor
NRs	nuclear receptors
NSCs	neural stem cells
PBS	phosphate buffered saline
PCAF	p300/CBP-associated factor
PCR	polymerase chain reaction
PNR	photoreceptor-specific nuclear receptor
PPAR	peroxisome proliferator-activated receptor
PRs	progesteron receptors

Abbreviation	Meaning
PXR	pregnane X receptor
RAR	retinoic acid receptor
RAR	retinoic acid receptor
RORα	RAR-related ophan receptor-α
RORβ	RAR-related ophan receptor-β
RXR	retinoid X receptor
S. mediterranea	*Schmidtea mediterranea*
SF1	steroidogenic factor 1
SMARC	SWI/SNF-related, Matrix-associated, Actin-dependent Regulator Chromatin
Smed-tlx-1	*S. mediterranea* homologue of nematode, insect and vertebrate genes *NHR-67, tailess and Tlx*, respectively- gene and/or transcript
SMED-TLX-1	*S. mediterranea* homologue of nematode, insect and vertebrate genes *NHR-67, tailess and Tlx*, respectively - protein
SMRT	silencing mediator of retinoid and thyroid hormone receptor
SRC	steroid receptor co-activators
SWI/SNF	SWItch/Sucrose NonFermentable
T3	triiodothyronine
T4	thyroxine
TIF2	transcriptional mediator/intermediary factor 2
tll	tailess, insect homologue of the chordate *Tlx*
tlx	tailles homolog
TR	thyroid hormone receptor
TRAM-1	thyroid hormone receptor activator molecule 1
UTR	untranslated region
VDR	vitamin D receptor

Abstrakt

Jaderné receptory zahrnují rozsáhlou rodinu transkripčních faktorů, které jsou silnými regulátory tkáňového metabolismu, homeostázy a vývoje tkáně živočišných druhů včetně člověka. Jsou zvláště zajímavé pro svoji schopnost reagovat na vyvázání hormonů, metabolitů, xenobiotik či uměle vytvořených molekul a převést interakci s těmito malými lipofilními molekulami do specifického regulačního signálu.

Při hledání jaderných receptorů, jejichž úloha by mohla být kritická pro nervovou tkáň u bezobratlých a zachovalá během vývoje živočichů, jsme identifikovali blízký homolog TLX obratlovců u ploštěnky *Schmidtea mediterranea*. Ploštěnky představují velmi slibný biologický model pro studium tkáňové homeostázy a regenerace. Ploštěnky jsou schopny vstřebávat vlastní tkáně a použít je jako zdroj energie během hladovění, a pomocí neoblastů znovu vytvořit celé svoje tělo při dostatku potravy. Informatická analýza veřejně přístupných dat sekvenovacího projektu *Schmidtea mediterranea* ukázala, že genom planarií obsahuje minimálně jeden gen s vysokým stupněm podobnosti s genem *tlx* obratlovců. Klonovali jsme kompletní CDS (coding DNA sequence of cDNA) a charakterizovali jsme gen funkčně. Ukázali jsme, že TLX (NR2E1) vykazuje u ploštěnky a obratlovců vysokou podobnost v jejich celé délce kódující sekvenci a odvozeného proteinu. Zjistili jsme, že TLX u ploštěnky, který jsme nazvali *Smed-tlx-1*, je exprimován v hlavičce, jakož i v kaudální části těla. Exprese *Smed-tlx-1* v hlavičce je přitom alespoň 10x vyšší ve srovnání s kaudální částí. Exprese *Smed-tlx-1* se po podání potravy zvyšuje přibližně dvakrát v obou těchto částech těla ploštěnky. Experimenty s RNA interferencí dále ukázaly, že *Smed-tlx-1* je kritický pro zachování stavby těla během cyklů hladovění/krmení a pro integritu oblasti mozku a očí ploštěnky.

V druhé části práce jsme se u glioblastomových buněčných linií zabývali expresí a distribucí TLX v buňkách. Zjistili jsme, že TLX se v buňkách nachází v několika formách proteinu, což svědčí o možných posttranslačních modifikacích TLX. V imunofluorescenční a kolokalizační studii jsme ukázali, že TLX je lokalizován do jádra i cytoplasmy a že jeho intracelulární distribuce může být regulována.

Naše výsledky ukazují, že funkce NR2E1 v homeostáze a vývoji nervové tkáně je evolučně zachovalá a že funkční mechanismus by mohl zahrnovat regulaci jeho intracelulární distribuce.

Klíčová slova

Astrocytom, buněčná reprodukce, diferenciace, homeostáza tkáně, imunofluorescence, NR2E1, *Schmidtea mediterranea*, TLX.

Abstract

Nuclear receptors constitute a large family of transcription factors that are powerful regulators of animal tissue metabolism, homeostasis, tissue maintenance and development. They are particularly attractive for their ability to respond to the binding of hormones, metabolites, xenobiotics and artificially prepared molecules and transmit the interaction with these small lipophylic molecules to specific regulatory potential.

In search for nuclear receptors that are likely to be critical for neural tissues in invertebrates and conserved during the evolution of animals, we have identified a close homologue of vertebrate TLX in a planarian *Schmidtea mediterranea*. Planaria represent very promising biological model systems for studies on tissue maintenance and regeneration. Planaria are able to resorb their tissues and use them as sources of energy during fasting and they re-build their bodies from neoblasts when food is plentiful.

Our search in *Schmidtea mediterranea's* publicly accessible genome sequencing data indicated that planarian genome contains at least one gene with a high degree of similarity to vertebrate TLX. We cloned full length CDS (coding DNA sequence of cDNA) and characterized the gene functionally. This showed that the planarian and vertebrate NR2E1 are highly similar in their entire coding sequence and the derived protein molecule. We found that the planarian TLX, that we name *Smed-tlx-1* is expressed in heads, as well as in tails of animals. *Smed-tlx-1* expression is at least 10times bigger in heads than in tails of animals and in both body parts increases approximately twice during the feeding phases. Inhibition of Smed-tlx-1 by RNA interference revealed that *Smed-tlx-1* is critical for sustaining the body plan during fasting – feeding cycles and for integrity of brain areas and eyes.

In the second part of the study, we studied the expression and intracellular distribution of TLX in glioblastoma cell lines. We have found that TLX is detected in multiple protein forms suggesting that they may be posttranslationally modified. Using immunofluorescence and collocalization studies, we show that TLX is localized in the nuclei as well as in the cytoplasm and we have found indications that TLX intracellular distribution may be regulated.

The results indicate that NR2E1 function in regulation of maintenance and development of neural tissues is evolutionarily conserved and its mechanism of function may include its regulated intracellular distribution.

Key words

Astrocytoma, cell reproduction, differentiation, immunofluorescence, NR2E1, *Schmidtea mediterranea*, tissue maintenance, TLX.

Introduction

Regulation network that controls cell fate and tissue maintenance including neuronal tissue is based on interaction of cell survival, renewal and differentiation stimuli (Reed 1999; Watt and Hogan 2000; Gage 2000; Pellettieri and Sanchez Alvarado 2007; Klein and Simons 2011) . Each of these steps is controlled by positive and negative stimuli that enable the organism to protect cells necessary for generation of new cells capable of sustaining the tissue integrity and directional programming of specific subpopulations of cells important for tissue functionality. Although the regulatory pathways of this process are known to a great detail, new regulatory molecules are being discovered by large scale studies.

Nuclear receptors (NRs), also termed nuclear hormone receptors (NHRs), are powerful regulators of animal development and metabolism. They are involved in regulation of specific cell functions as well as integration of developmental and metabolic processes at the level of organism. Their structure contains highly conserved DNA binding domain (DBD) that is coordinated by two zinc ions in a form consisting of two “zinc fingers” and a carboxy terminal ligand binding domain (LBD) composed of 12 helices. . Currently, for the description of nuclear receptors the classification into 5 main domains is used (Krust et al. 1986; Kumar et al. 1986; Kumar et al. 1987; Danielsen et al. 1987; Evans 1988; Ruff et al. 2000; Germain et al. 2006): N-terminal domain, DBD, hinge domain, LBD, C-terminal domain. Several members of nuclear receptor (NR) family are hormonal receptors, such as steroid hormone and thyroid hormone receptors, retinoid receptors and Vitamin D receptor. A growing number of NR family members are recognized as receptors capable to bind small molecules that, dependent on the receptor-ligand binding affinity modulate the receptor transcriptional functions (Antebi 2006; Kininis and Kraus 2008; McEwan 2009).

Ligands with very big affinity that fulfill the criteria of hormonally active compounds, usually derived from metabolites or molecules obtained with food, execute regulatory functions at the local or tissue restricted level as well as at the level of entire organism (Jacobs and Lewis 2002) . Specific ligand binding properties of many NRs evolved during evolution. At the same time, a ligand does not exist or is not known so far in the case of

many nuclear receptors (Giguere et al. 1988; Mangelsdorf and Evans 1995; Horard and Vanacker 2003). These receptors are known as orphan nuclear receptors.

The superfamily of NRs includes members that are highly conserved in distant animal phyla as well as receptors that apparently diversified (Escriva et al. 2000; Robinson-Rechavi et al. 2003; Escriva et al. 2004). Some NRs, like RARs related HR3 NRs seem to have similar functions in insects and nematodes while other including the multiplied HNF4 related NRs in *C. elegans* seem to have acquired new functions (Antebi 2006).

Another class of NRs, RXRs are conserved between fungi, *Cnidaria* and vertebrates, but their orthologue is missing in many nematode species (Antebi 2006). In case of RXR, the ligand binding specificity, its DNA binding specificity and dimerization capabilities are conserved between *Cnidaria* and vertebrates (Kostrouch et al. 1998).

It can be speculated that the regulatory network governing the reprogramming of cells in various tissues include growth factors, cytokines and timing, and that the specification directing network is based on NRs and their ligands. This network is likely to be complex and include several coherently functioning factors. For its analysis, systems that are efficient and inexpensive are necessary. Such systems are represented by invertebrate model organisms that may allow visualization of conserved regulatory mechanisms that may be tested later on vertebrate models.

Rationale behind the dissertation

Nuclear receptors are important transcription factors that show both enormous variability and conserved features. While the variable features are likely to be phyla of genus specific, the conserved are likely to reflect core mechanisms that govern the very basic regulatory pathways of Metazoan cells (Sluder and Maina 2001; Enmark and Gustafsson 2001).

Combination of classical biology, genetics and genomics can put a new light on data that seem to be petrified. This is especially true for the rapidly growing genome sequencing projects that are revealing data accessible to the computer meta-analysis.

One such model is the model of *Schmidtea mediterranea*. *S. mediterranea* is a flatworm, platyhelminth. Many platyhelmints are dangerous parasites, difficult to control and causing suffering of animals including modern as well as ancient man (Eckert et al. 2000; Olson et al. 2001). But non-pathogenic flat worms of genus *S. mediterranea* becomes a very powerful model organism for its capabilities to regenerate tissues and especially to re-grow entire organism from small fragments of body (Newmark and Sanchez Alvarado 2002; Salo 2006; Baguna 2012). This requires complex rearrangement of tissues and restoration of new body plan from multipotent or totipotent cells, the neoblasts (Sanchez Alvarado et al. 2002; Cebria et al. 2007; Wenemoser and Reddien 2010; Wagner et al. 2011). This capability of regression and re-growing is also taking place in adaptation of the animals to critical food restriction (Nimeth et al. 2004; Pellettieri et al. 2010; Gonzalez-Estevez et al. 2012).

These animals are able to sustain their existence under starvation (during which the number of cells and the animal size are decreasing) by utilization their own tissues as the energy supply while protecting the pluripotent cells that are able to support the complete re-growing of animals when the food supply is restored (Baguna and Romero 1981; Romero and Baguna 1991; Oviedo et al. 2003; Nimeth et al. 2004; Pellettieri et al. 2010; Fraguas et al. 2011; Gonzalez-Estevez et al. 2012). This process requires a complex regulatory network that sacrifices some cells while protects other (for detailed reviews concerning *S. mediterranea* see Newmark and Sanchez Alvarado 2002; Salo 2006; Pellettieri and Sanchez Alvarado 2007; Sanchez Alvarado 2007; Baguna 2012).

As shown in the first part of this dissertation, we were able to show that the nuclear receptor Smed-TLX-1, a homolog of TLX in vertebrates, plays the important role in a regeneration of *S. mediterranea*. This finding immediately turned our attention to vertebrates including humans. Namely, recent studies have indicated that brain stem cells in vertebrates depend critically on a transcription factor TLX, that belongs to the superfamily of nuclear hormone receptors (Shi et al. 2004; Qu and Shi 2009; Chavali et al. 2010). In the second part of this dissertation, we have therefore chosen the model of human glioblastoma (astrocytoma) cells as several lines of evidence also indicated that vertebrate TLX promotes the tumorigenic potential of glial cells, and that TLX is expressed in astrocytic tumors and glioblastoma cell lines (Liu et al. 2010; Park et al. 2010). Its expression was also linked to worse prognosis of patients with malignant astrocytic tumors (Park et al. 2010).

TLX (NR2E1) nuclear receptor

TLX belongs to the group of orphan nuclear receptors. It was first identified in the neuroepithelial embryonic tissue firstly in chicken, and immediately also in mice to confirm its universal presence in vertebrates (Yu et al. 1994). This protein showed strongest homology with the insect tailless (TLL) receptor in Drosophila melanogaster where the amino acid sequence comparison indicated 60% similarity in the DBD region and 41% similarity in LBD region. Comparison with other to that date known nuclear receptors, as EAR3/COUP, RXRalfa and RARalfa did not exceed 60% similarity for DBD and 41% similarity for LBD (Yu et al. 1994). Interestingly, *tlx* similarly as earlier cloned *tll* (Pignoni et al. 1990) differs from the rest of the nuclear receptors especially in the P and D boxes of the DBD domain. In both receptors, in P box normally present lysine is replaced by a different amino acid and in the D box, which in the rest of the nuclear receptor superfamily normally consists of 5 amino acids, are 2 amino acids extra, making a total of 7 amino acids per P box (Yu et al. 1994). Even more striking evidence of homology was the result of *in vivo* test, where ectopic expression of vertebrate TLX in fly embryos could perfectly mimic the function of Tll (Yu et al. 1994).

In developing mice the *tlx* gene is expressed in telencephalon and dorsal midbrain and also in the optic cups and nasal placodes (Monaghan et al. 1995). Even more convincing argument to think about TLX in connection with central nervous system (CNS) was, that although artificially *tlx* knock-out mice were able to develop and live, they displayed serious changes in behavior, i. e. increased aggressiveness for both genders although more serious for males (even killing their littermates), lack of maternal instincts in the female cases, and also seriously impaired spatial learning abilities (Monaghan et al. 1995; Roy et al. 2002). In correspondence with the behavior and memory deficits, anatomical studies of the mutant mouse reported decreased sizes of the rhinencephalic and limbic structures (Monaghan et al. 1997; Roy et al. 2002) and also reduction of neocortical thickness by 20% (Land and Monaghan 2003). Similarly same defects in mouse behavior and brain malformations were also observed in cases of spontaneous *tlx* mutations (Young et al. 2002). All these newer findings were more and more supporting the original prediction that TLX is very probably essential for proper proliferation or survival of certain subpopulations of neural progenitor cells in these affected regions of CNS (Monaghan et al. 1997).

But what were these subpopulations? The most important step to answer this question started by the identification of mammalian adult neural stem cells (NSCs) (Eriksson et al. 1998; Doetsch et al. 1999). This naturally led to a search for different regulation pathways which would keep these cells renew and keep their pluripotent potential. And finally laboratory of Shi pointed to TLX as to a very promising and crucial regulator of adult neural stem cells renewal and preservation (Shi et al. 2004). The reasons to point to TLX were several. First, thanks to β-galactosidase reporter artificially knocked into the *tlx* locus the expression of *tlx* could be visualized through LacZ staining. This pointed out locations in dentate gyrus and in subventricular zone which are also places where adult NSCs typically reside (Eriksson et al. 1998; Doetsch et al. 1999; Shi et al. 2004). Moreover colocalization of β-galactosidase and nestin, a marker of proliferation CNS progenitors indicated TLX expression in adult NSCs or progenitor cells (Shi et al. 2004). Next, TLX expressing cells isolated from the normal mice adult brains could proliferate, self-renew, have pluripotent neuronal potential and expressed nestin. In contrast *tlx* null cells from the mutant mice could not proliferate and expressed GFAP, an astrocyte marker (Shi et al. 2004). Moreover successful rescue of *tlx* null cells was done through infection with lentiviral vector expressing TLX. As the result, infected cells regained their ability to proliferate and express nestin again (Shi et al. 2004).

The laboratory of Shi convincingly proved by means of several different approaches that TLX directly represses expression of astrocyte specific genes and that possibly transcriptional repression is crucial in preserving the undifferentiated state of neural stem cells (Shi et al. 2004).

The role of TLX as primarily transcription repressor was in correspondence with older (Yu et al. 2000) and also subsequent studies which pointed out interaction of TLX with co-repressors such as atrophin1 (Atn1) (Zhang et al. 2006; Estruch et al. 2012), HDACs (Sun et al. 2007) and lysine-specific demethylase 1 (LSD1) (Yokoyama et al. 2008).

The original *tlx* expression studies (Yu et al. 1994; Monaghan et al. 1995) suggested that TLX has probably very important function also in the visual system. This was confirmed by Yu et al. (2000). The authors proved, while using *tlx* -/- mice, that TLX is a key component of retinal development and vision (Yu et al. 2000). TLX in mice is expressed in retinal

progenitor cells and is also present in two types of eye glial cells, Muller cells and astrocytes. TLX appeared to be critical for the maturation of these cells (Miyawaki et al. 2004). Moreover TLX has been shown to be required for proper coordination of retinal proliferation and differentiation to prevent retinal dystrophy (Zhang et al. 2006). Strikingly, experimental knock in of bacterial artificial chromosome carrying single copy of human *tlx* completely restored (evaluated by retinal histology and electroretinograms) the retinas of *tlx* null mice. Interestingly enough, no such effect was observed on other CNS deformities (Schmouth et al. 2012).

No wonder that this intimate relation between TLX and adult NSCs proliferation and renewal lead to speculations that TLX could play an important role in tumor development. And indeed, it was demonstrated that neural stem cell-specific overexpression of TLX in transgenic mice leads to neural stem cell expansion and glioma-like lesions in aged mouse brains (Liu et al. 2010). If this overexpression is also combined with p53 knock out, then these lesions typically progress to invasive gliomas. In addition, the expression study with human malignant astrocytomas was performed. Its results revealed that in 9 cases out of 41 primary glioblastomas the expression of TLX was increased (Liu et al. 2010). Another study reported increased mRNA TLX levels in 5 out of 7 human glioma cell lines and also increased TLX mRNA levels in 2 out of 6 glioma stem cells derived from patients with gliomas (Park et al. 2010). Moreover, the data analysis of 297 glioma patients taken from the Repository of Molecular Brain Neoplasia Data (REMBRANDT) database of the National Cancer Institute revealed correlation of increased TLX expression levels and poor survival prognosis suggesting TLX as a possible diagnostic marker (Park et al. 2010).

Specific Aims

Following the identification of partial sequence of NR2E1/TLX in *Schmidtea mediterranea*, we aimed at cloning full length cDNA and characterization of *S. mediterranea* NR2E1 functionally in the study entitled "Search for factors with regulatory potential on neuronal reprogramming and regeneration using an invertebrate model of *Schmidtea mediterranea*", or shortly "Search for TLX (NR2E1) in *Schmidtea mediterranea*" (Part I). Next we attempted to study NR2E1 in human astrocytic cell lines at the cell biology level to establish its expression pattern in the study entitled "Expression of TLX (NR2E1) in human astrocytoma cell lines." (Part II).

Specific hypotheses

NRs represent a very interesting group of transcription factors that can be influenced by small pharmacologically accessible compounds. NRs are typically expressed in large variety of cell types yet regulate very specific developmental and metabolic processes. Numerous examples document a potential of NRs to accept new roles during evolution of Metazoa. Some core mechanisms of function of NRs are conserved and can be identified in distant organisms. Conserved mechanisms are more likely to be functionally important than mechanisms that are genus, or phyla specific. Finding of pathways that are conserved for NRs may be of great importance.

NRs are transcription factors found only in Metazoa. They are not found in yeast. They are present in Cnidaria (jellyfish, corals and anemones), sponges and all animals higher in the evolutionary tree that were studied for the existence of NRs.

Since *S. mediterranea* genome is partially sequenced and accessible online, we decided to search for conserved NRs.

We hypothesized that some mechanisms of NRs may be conserved from Platyhelminthes to man, and accessible for a focused study.

Materials and Methods

Part I: Asexual strain of S. metiterranea was maintained under standard conditions), and the following methods were explored: isolation of RNA, cRNA preparation, reverse transcription, RT-PCR, cloning and sequencing, qPCR, RNA interference, in vivo imaging, informatics and modeling (Raska et al, 2011).

Part II: Well characterized 4 glioblastoma cell lines were cultured under standard conditions. Western blots from the cellular extracts were performed in which 2 antibodies to TLX were essayed. For imunocytochemical localization, strandard processing procedures were employed. Besides anti-TLX antibodies, antibodies against MSM2 protein and gamma tubulin as well as DAPI staining were used in order to perform colocalization experiments. Images taken in the Olympus AX-70 microscope were processed by image analysis.

Results and Discussion (Synopsis)

The work that constitutes this thesis is a part of the effort to contribute to the elucidation of mechanisms by which NRs regulate metabolism, tissue homeostasis and development. The work presented in this thesis represents an attempt to identify conserved mechanisms that may be critical for regeneration and maintenance of neural tissues. These mechanisms are likely to be important not only at normal or optimal conditions of the organisms' development and function but also at pathological conditions involving wound healing, neurodegenerative diseases, and tumors.

Nuclear receptors constitute a large family of transcription factors including receptors that regulate their target genes in response to binding of specific ligands, hormones, metabolites and xenobiotics. They are expressed in a large variety of cell types at various levels and constitute a regulatory network including nuclear receptors, transcription cofactors, ligands and interact with other regulatory pathways including transduction signaling pathways and metabolic pathways.

In search for NRs that are likely to be critical for neural tissues, we have chosen planaria for our research. Planaria have been used as classical biological systems from the earliest years of systematic biological research. Contemporary development of genetic and genomic tools brings this model again into the focus of modern biology and makes it especially attractive for studies on regeneration and tissue maintenance. The genome sequencing project that is currently underway on *Schmidtea mediterranea* is providing enormous wealth of data that may speed up the research on this organism (Nagasawa et al. 1997; Newmark and Sanchez Alvarado 2002; Salo 2006; Pellettieri and Sanchez Alvarado 2007; Fernandes and Sternberg 2007; Sanchez Alvarado 2007; DeMeo et al. 2008; Ririe et al. 2008; Kato and Sternberg 2009; Sarin et al. 2009; Chavali et al. 2010; Baguna 2012). Our search for NRs in *Schmidtea mediterranea* indicated that their genome contains close homologues to most vertebrate NRs. However, many receptors seem to be divergent in their structure and function. With respect to neural tissue, the predicted closest homologue of vertebrate TLX seemed to be especially well conserved in a part of its predicted sequence but diverged at both N and C termini of the predicted derived proteins. This would suggest a diverged functionality of *S. mediterranea* TLX.

The structure of the DNA binding domain of NR2E subclass of NRs differs from all other NRs by including additional amino acids. This is not an exception in the evolution of Metazoan species. For example, *Platyhelminthes* have NRs similar to thyroid hormone receptors that have two DNA binding domains in their molecule, a situation that is not found in vertebrates. The sequence of NR2E NRs in *C. elegans* and in *Drosophila* seems to be divergent from the vertebrate counterparts and differs also in the overall size of the molecule. On the other hand, the DNA binding domain of NR2E NRs seems to be conserved between various Metazoan phyla indicating that this receptor evolved before these phyla separated during Evolution.

To learn more about the TLX in *S. mediterranea*, we decided to clone its mRNA and characterize it functionally. This led to finding that *S. mediterranea* has a surprisingly close homologue to vertebrate TLX. Expression analysis indicated that *Smed-tlx-1* is expressed at both ends of the planarian body, in heads and in tails, although about 10 times bigger expression was detected in heads of animals that contain primitive brains, compared to tails.

Planaria have specialized cells, called neoblasts that have the potential of stem cells for all planarian cell types. These cells are able to reproduce and rebuild the complete planarian body plan from small fragments. They are also able to serve as the pool of progenitors for growing the planarian tissues. *Planaria* are able to use their bodies as energy sources in a regulated way during fasting and they re-grow from neoblasts at favorable conditions organism (Nagasawa et al. 1997; Newmark and Sanchez Alvarado 2002; Salo 2006; Pellettieri and Sanchez Alvarado 2007; Fernandes and Sternberg 2007; Sanchez Alvarado 2007; DeMeo et al. 2008; Ririe et al. 2008; Kato and Sternberg 2009; Sarin et al. 2009; Chavali et al. 2010; Baguna 2012). Our expression analysis showed that the expression of *Smed-tlx-1* is augmented during feeding periods compared to fasting. This indicated that Smed-TLX-1 may be more important for growing periods then during the animal regression. Inhibition of *Smed-tlx-1* by RNAi revealed its function during the growing period in feeding-fasting cycles and a role for development of neural tissues and eyes.

This part of the study indicated that the developmental role of NR2E is likely to be evolutionarily conserved.

In the second part of the study, we attempted to expand the knowledge of the NR2E function in the model of human glioblastoma cells.

TLX was shown to be a strong repressor required for maintenance of neural stem cells. On the other hand, the role of vertebrate TLX for activation of transcription of specific genes was also shown. We have chosen the model of human glioblastoma/astrocytoma cells as several lines of evidence also indicate that vertebrate TLX promotes the tumorigenic potential of glial cells, and that TLX is expressed in astrocytic tumors and glioblastoma cell lines. Its expression was also linked to worse prognosis of patients with malignant astrocytic tumors.

Astrocytes are formed from the common neural progenitors – neural stem cells representing a cell type with features known for mesenchyme. They have potential of multiplication and remodeling of brain tissue and wound healing. This feature gives them in their malignant variant an extreme malignant potential and a tendency of fast spreading.

Using Western blot analysis and two antibodies designed to recognize different domains of the protein, we have found that TLX is expressed in human astrocytic cell lines at various levels and may be posttranslationally modified. The existence of posttranslational regulation of TLX was already proposed (Obernier et al. 2011).

Immunofluorescence analysis detected human TLX in nuclei as well as in the cytoplasm. Immunofluorescence and correlation to the staining of DNA by DAPI or the expression of minichromosome maintenance protein 2 (MCM2) (Masata et al. 2011a) revealed that the ratio between nuclear and cytoplasmic TLX differs between individual cells and during particular phases of the cell cycle as shown by colocalization with MCM2 and gamma-tubulin antibodies (Azimzadeh and Bornens 2007; Masata et al. 2011b). The cells of individual cell lines also showed a different predominant pattern of TLX staining. This indicated that TLX moves between the nucleus and the cytoplasm and its distribution may be regulated. TLX signal greatly increased in the extrachromosomal compartment during mitosis suggesting a possibility that TLX is efficiently synthesized during G2 and/or TLX molecules bound to chromatin are released from chromatin during chromosome compaction. In agreement with the findings of (Maruvada et al. 2003), our results suggest that TLX function is further regulated by intracellular distribution.

Conclusions

Based on the work presented in this thesis, we conclude that:

NR2E1 function in regulation of neural tissues is evolutionarily conserved from planaria to man.

Smed-TLX-1 regulatory function is revealed in proliferative phases of tissue growth.

hTLX shows signs of translocations between the nucleus and the cytoplasm suggesting regulated intracellular distribution and the regulation of TLX on the protein level.

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hTLX shows signs of translocations between the nucleus and the cytoplasm suggesting regulated intracellular distribution and the regulation of TLX on the protein level.

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