EXPRESSION OF THE DTK RECEPTOR TYROSINE KINASE DURING ZEBRAFISH DEVELOPMENT

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The activation of receptor tyrosine kinases (RTKs) following ligand binding plays a key role in many developmental processes (Schlessinger and Ulrich, 1992). Dtk (Crosier et al., 1994) belongs to a subfamily of receptor tyrosine kinases which includes Axl (O’Bryan et al., 1991) and Mer (Graham et al., 1994). Members of this subfamily are structurally characterized by the presence of two immunoglobulin-like domains juxtaposed with two fibronectin type III domains in their extracellular regions. Both Dtk and Mer are highly expressed in the adult brain. In the rat, Dtk expression increases during late embryonic neuronal development and remains at high levels postnatally (Lai and Lemke, 1991). Other adult mouse tissues which express significant levels of Dtk include testis, ovary, lung, bladder and portions of the gastrointestinal tract. In contrast to its restricted expression in adults, the Dtk gene is widely expressed during embryonic development (Crosier et al., 1994). Dtk ligands have been identified as the protein encoded by the growth-arrest-specific gene (Gas6) and the anticoagulation factor protein S (Stitt et al., 1995; Mark et al., 1996), both belonging to the vitamin K-dependent protein family.

The zebrafish is an excellent system for investigating the function of genes involved in embryonic development, especially in the central nervous system (CNS) (Kimmel, 1993). A cDNA that encodes the zebrafish homologue for the Dtk receptor tyrosine kinase has been isolated and sequenced, showing overall 48% amino acid identity with the human counterpart and 72% identity in the region of the kinase domain (Walshe et al., submitted).

We have undertaken the study of the expression pattern of Dtk during zebrafish embryonic development using different approaches. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis using specific oligonucleotides for Dtk transcripts was carried out on total zebrafish RNA and the specificity of the amplified products was confirmed by hybridization to probes containing sequences internal to the primers. Dtk expression could be detected from the beginning of gastrulation through the segmentation period, as well as in whole embryos polymerase chain reaction (RT-PCR) analysis using specific oligonucleotides for Dtk transcripts were analysed by Northern blot using a CDNA probe that contained sequences encoding part of the extracellular domain of Dtk. Using this technique, the full length transcript was only detected in adult, suggesting that the level of expression is higher in this period.

To gain insight into the pattern of expression of this receptor during the embryonic development of zebrafish, whole-mount in situ hybridization using Digoxigenin-labelled RNA probes corresponding to different regions of Dtk have been performed. Zebrafish embryos were grown in Phenyl thiouracil (PTU) treated water to inhibit the development o pigment. The in situ hybridization method was based on that of Harland (1991) for Xenopus with several modifications. As observed by RT-PCR analysis, Dtk expression was first detected during early gastrulation in the zebrafish embryo. By the end of gastrulation Dtk transcripts were detected throughout the embryo. During the early segmentation period low levels of Dtk expression were detected in the brain and by 48 hours post fertilization Dtk expression appeared to be localized to posterior borders of the telencephalon, anterior regions of the midbrain, and discrete cells within the hindbrain.

In order to locate more accurately the sites of expression, we have undertaken comparative whole-mount in situ hybridizations with genes which are expressed in discrete domains of the zebrafish brain during the pharyngula period (24-48 h). These include Distal-Less 2, expressed near the ventricular surface of the telencephalon (Akimenko et al., 1994) and hlx-1, which is expressed in reticulospinal interneurons defining rhombomere boundaries (Fjose et al., 1994). These results suggested a correlation with the areas of expression of Dtk, although two-color whole-mount RNA in situ hybridization is necessary to precisely determine the cells containing the receptor.

Our results provide insights into the dynamic expression patterns which exist for Dtk during zebrafish development and extend knowledge of the regions in the brain which express Dtk. Further studies including perturbation of Dtk expression in zebrafish embryos in conjunction with identification of receptor ligand(s) for this species will contribute to the understanding of the role of Dtk in brain development.

References


