1. Introduction

1.1. Pituitary tumor etiology

The pituitary gland forms different benign tumors (adenomas), some of which secrete one or several hormones of the normal anterior pituitary gland, while others do not. The exact aetiology of these adenomas remains largely unknown. Somatic mutations, which are characteristic of human malignancies, have not been identified yet. Single base substitutions in the Gs protein have been implicated in the pathogenesis of some growth hormone (GH) producing somatotroph adenomas (1), but these mutations are present in only a minority of adenomas.

The expression of any single subtype of these adenomas varies enormously between individual patients. Adenomas may grow slowly or aggressively; they may secrete very small or very large amounts of a hormone or produce but not secrete a hormone, or they may not produce any hormone at all. The exact mechanisms that underlie these differences are largely unknown.

1.2. Growth factor actions in the normal and tumorous pituitary

The normal pituitary gland secretes a number of growth factors and cytokines (from here on both are referred to as growth factors), which represent potential candidates influencing normal pituitary development and characteristics (2). Epidermal growth factor (EGF), transforming growth factor-α or -β (TGF-α, -β), basic fibroblastic growth factor (bFGF), insulin-like growth factor 1 (IGF1), interleukin-2 and -6 (IL2, IL6) are known to modulate hormone production or cell growth in normal cells. They have been demonstrated in pituitary tumors (3, 4, 5, 6) and some of them can modulate hormone secretion or growth of pituitary adenomas *in vitro*. Some of the better-known growth factors, which have been implicated to exert effects in permanent or primary pituitary cells, are described in the following chapter.

bFGF can stimulate prolactin (PRL) and GH secretion in pituitary adenoma cell cultures, but does not have an effect on adenoma cell proliferation *in vitro* (7).

TGF-α and its receptor have been identified in rat pituitary tumors (8) and normal cells (9) and they appear to be necessary for tumor expression (8). In a transgenic

mouse model, in which TGF-α was over expressed using the PRL-promoter, the mice developed pituitary adenomas, suggesting a role of TGF-α in tumor development (10).

EGF is a potent mitogen for normal pituitary cells. It can up regulate ^{3}H-thymidine uptake in pituitary cells from non-functioning tumors (NFT) and its receptor is over expressed in those tumors (11). The EGF receptor has been detected in normal pituitary and in some functional and non-functional adenomas with extremely variable intensity. The highest degree of EGF receptor mRNA was present in somatotroph adenomas and the aggressive silent subtype 3 adenomas (12).

IGF1 has been shown to inhibit GH secretion and stimulate PRL secretion in cultured pituitary adenomas, but does not have an effect on their proliferation (13, 14, 15). In contrast, the growth of rat pituitary tumor cells can be up regulated by this growth factor (16).

1.3. Primary aim of the project

The primary aim of this project was to characterize potentially important growth factors and to further elucidate their role in the development and differentiation of human pituitary adenomas. A systematic study of the characteristics of known growth factors was undertaken and compared to characteristics of fractionated extracts from human pituitary adenomas. This included assessing the effects of growth factors and tumor fractions on hormone secretion (GH and PRL), as well as on cellular growth (^{3}H-thymidine incorporation, cell counts, apoptosis).

1.3.1. Description of a rat pituitary tumor cell line

Since primary human pituitary adenoma cells are difficult to maintain in long-term culture and have a very low proliferation rate, most of the experiments were carried out in GH_3 cells. This cell line is derived from a pituitary tumor in Wistar rats and has since been well described (17). The cells secrete GH and PRL and can be easily held in long-term culture (18).

1.3.2. Primary cultures of human pituitary adenomas

Primary human adenoma cells, which were generated from human pituitary adenomas of various subtypes and maintained in culture for up to 14 days were used to verify the results obtained with the permanent tumor cells and to further study potential mechanisms for growth factor regulated pituitary tumor growth.

1.4. Structure and function of IGF1 and its receptor

IGF1 was of particular interest in this study. The IGF-system comprises two ligands, two receptors, IGF binding proteins (IGFBP) and IGFBP-specific proteases. IGF1 is a single chain peptide with a molecular weight of about 7.6 kD. The Type I IGF receptor (IGF1R) is a tetrameric molecule, consisting of 2 extracellular alpha-subunits and two transmembrane/intracellular beta-subunits, which are linked by disulfide bonds. The IGF1R belongs to the tyrosine kinase family, binds IGF1 with high affinity, has 2-3 times lower affinity for IGF2, and approximately 100 times lower affinity for insulin (19, 20).

The most important physiological role of IGF1 is that of a primary growth regulator. Blockade of ligand-mediated signal transduction through the IGF1R using a monoclonal antibody inhibits tumor growth in many model systems (21, 22). The IGF1R is also required for optimal growth in vivo and in vitro (23). A decrease in the IGF1R causes massive cell death in vivo, as shown with antisense transfection experiments (24). Embryos from mice with a targeted disruption of the IGF1R are 45% of normal size and nonviable at birth (25). In contrast, activation of an over-expressed IGF1R initiates mitosis (23). The IGF1R is thought to control the progression from G1 into S-phase of the cell cycle (23, 20). Under-expression would prevent the G1 to S-phase transition. The IGF1R is also obligatory for the establishment and maintenance of the transformed phenotype for several types of cells. In the presence of ligand transformation occurs when the IGF1R is over-expressed in mouse fibroblasts (26).

In general, there is a close relationship between cell cycle progression and apoptosis. Apoptosis is promoted by cell cycle arrest. It is likely that apoptosis and mitosis share biochemical pathways because the morphologic features of both processes include chromatin condensation, loss of cell-cell contact, and nuclear

disintegration (23). Singelton et. al have shown that IGF1R activation maintains normal Bcl-2 levels, which encode a protein that suppresses naturally occurring cell death. IGF1R activation also prevents ICE/LAP-3 processing, which is a protease present in apoptotic cells. And at last, increased IGF1R expression enhances the negative death regulator Bcl-xl (27).
The receptor transduces its signal via autophosphorylation of tyrosine residues and subsequent tyrosine phosphorylation of downstream targets, including insulin receptor substrate-1 (IRS-1), IRS-2, Shc, and Crk (23). The concluding step in the antiapoptotic signalling is the phosphorylation, by Akt/protein kinase B, of BAD, one of the members of the Bcl-s family of proteins (28).

1.5. Secondary aim of the project

At present, this complex system is only poorly characterized in pituitary adenomas. Although IGF1 is an important mitogen in many tumor types and normal cells, and is considered primarily mitogenic (29), only its metabolic effects have been demonstrated in pituitary adenomas. *In vitro*, it is mitogenic for human gonadotropinoma cells (30), but not for somatotropinoma cells (31). To further elucidate the role of IGF1 and its receptor in pituitary tumor development and differentiation was the second major goal of this study. It involved studying the role of this growth factor and its receptor in apoptosis of primary human pituitary tumor cells.

This work also involved a number of methodological refinements, as well as the development of a method to determine of cell surface IGF1R expression. In the main section, data on these experiments are described first. Then, the influence of growth factors and tumor fractions on GH_3 cells are portrayed. Finally, experiments with primary human pituitary tumor cells are documented.