Chapter 1

General introduction
GENERAL INTRODUCTION

Von Hippel Lindau (VHL) disease is an autosomal dominant syndrome leading to the onset of benign and malignant tumours in multiple organs. The cause of this disease is loss-of-function of the VHL tumour suppressor gene (1). With an incidence of 1 in 36,000 to 85,000 newborns, VHL is a rare disease (2). The morbidity associated with VHL-related manifestations is high. Mortality in VHL is most often due to the vascularised haemangioblastoma, with permeable vessels causing oedema and obstructive hydrocephalus and due to the metastasising renal cell carcinoma (1, 3). The vascularised nature of the VHL-related manifestations is a hallmark of the disease and directly linked to the absence of normal VHL protein (4-9) (see Fig. 1). A defect or absent VHL protein causes an accumulation of hypoxia inducible factor (HIF). HIF will, if not degraded by VHL protein, lead to downstream signalling with transcription of vascular endothelial growth factor (VEGF-A) and chemokine receptor 4 (CXCR4). VEGF-A is a potent angiogenesis initiating factor and is highly expressed in most tumours (10, 11). CXCR4 is a G-protein coupled chemokine receptor. The pivotal role of CXCR4 and its ligand (CXCL12) in the proliferation of tumour cells, induction of angiogenesis, invasive tumour growth and metastasis formation is well known. In both a preclinical breast cancer mouse model as well as in breast cancer patients the CXCR4/CXCL12 axis plays a critical role in the determination of the metastatic destination of tumour cells. Neutralising CXCL12 with antibodies blocking CXCL12 interaction with CXCR4, inhibited metastasis formation in a human tumour bearing mouse model (12).

VHL-mutation carriers can be grouped according phenotypic appearance of disease. The exact relation between genotype and VHL phenotype is as yet unclear (13). The VHL phenotypes are distinguished by their risk of development of pheochromocytomas and renal cell carcinomas. Phenotype 2a and 2b include VHL-mutation carriers with risk to develop all VHL-related manifestation with for 2a a low risk and 2b a high risk of development of renal cell carcinoma. Phenotype 2c includes carriers with only pheochromocytomas. Phenotype 1 carriers do not develop pheochromocytomas but harbour a risk to develop all other VHL-related manifestations (14). VHL surveillance guidelines do not differentiate between phenotypes. Because of the yet unpredictable clinical behaviour of VHL-disease and the in potential life threatening consequences, intensive and lifelong surveillance is recommended.
Figure 1 | VHL-dependent HIFalpha pathway
Under normoxic condition pVHL binds to HIFalpha, which causes subsequently ubiquination of the HIFalpha subunit, followed by proteosomal degradation. Under hypoxic conditions the pVHL can no longer bind to HIFalpha. This leads to downstream transcripion of a.o. VEGFA, CXCR4, CXCL12 by activation of the hormone response element (HRE) within the promoter after binding of HIFalpha to its beta subunit. In case of a VHL gene mutation (or hypermethylation of the VHL gene promotor) the pVHL does not work properly causing HIFalpha binding to HIFbeta and downstream signaling under all condition (normoxic and hypoxic). This figure was created with Servier Medical Art.

Aim of the thesis
The aim of this thesis is to elucidate clinical features and shed more light on the behaviour of VHL-disease. In addition, the role of CXCR4 in VHL-disease and VEGFA in AML is studied.

Outline of the thesis
In VHL-disease a congenital mutation in the VHL-gene leads to a defect VHL protein resulting in enhanced transcription of a.o. VEGF-A and chemokine receptor CXCR4. Several in vitro and in vivo models have revealed the key role of the chemokine receptor CXCR4 and its ligand CXCL12 in tumour biology. High levels of CXCL12 protein stimulate proliferation and migration of CXCR4-expressing cancer cells. In Chapter 2 we aimed to provide an overview of the structure, activation and downstream signaling pathways and the function of different chemokines and their receptors in cancer and non-cancer setting. Knowledge of the function of the CXCR4/CXCL12 axis under physiological circumstances supports insight in the consequences of the upregulation of this axis in VHL-disease. In Chapter 3 we aimed to provide an overview of the role of CXCR4 and its
ligand CXCL12 with a special focus on drugs targeting this axis to improve treatment results. In this chapter we also summarize preclinical studies of breast cancer, glioblastoma and neuroblastoma involving CXCR4. These studies show that recruited CXCR4-positive monocytes in the tumour release amongst others VEGF-A. These monocytes together with the bone marrow derived endothelial and pericyte progenitors will induce new vessel formation. Excessive vessel formation is also one of the hallmarks of VHL-disease.

The VEGF-A family can be subdivided in multiple isoforms, named after the amino acid length of the corresponding protein of each VEGF-A isoform. Earlier studies have correlated total VEGF-A to worse prognosis in paediatric AML samples (15, 16). The distinct characteristics of an isoform are decisive for the pattern of angiogenesis (the freely diffusible isoform VEGF121, intermediate diffusible VEGF165 and extra cellular matrix bound VEGF189 and VEGF206) which might be important for disease outcome and response to therapy. Therefore, in Chapter 4 the expression of different isoforms of VEGF-A in relation to clinicopathologic characteristics and outcome and the co-expression of these isoforms was analysed. We determined the VEGF121, VEGF145, VEGF148, VEGF165, VEGF183, and VEGF189 mRNA expression levels in paediatric AML patients. In this study samples of 30 children obtained at time of AML diagnosis were included. After isolation of mRNA from purified AML cells and preparation of cDNA, an qRT-PCR was performed. Statistical analyses were used to compare expression levels and outcome, clinicopathologic characteristics and correlations amongst different isoforms.

Central nervous system haemangioblastomas are vascularised tumours occurring both in patients with von Hippel-Lindau disease as well as sporadically. Biallelic inactivation of the VHL-gene has been found in 62-66% of VHL-disease related haemangioblastomas. In sporadic haemangioblastomas somatic inactivation and biallelic inactivation of the VHL-gene is found in 20-50% and 0-13% of cases respectively. The downstream consequences of a likely difference in molecular pathways between VHL-related and sporadic haemangioblastoma are as yet unclear. Our main objective in Chapter 5 was to study the effect of a VHL-mutation on downstream VEGF-A, CXCR4 and CXCL12 protein expression in VHL-related and sporadic haemangioblastomas and correlate the expression levels to preoperative size of the lesions. Of all patients operated in the UMC Groningen for haemangioblastomas during the years 1995-2010 the available freshly frozen tissue was collected. In total 33 surgical specimens derived from 27 patients were retrieved, of which 11 patients were diagnosed with VHL-disease and 16 patients with sporadic haemangioblastoma. Immunohistochemical analysis of 33 haemangioblastoma specimens was performed for haematoxyline and eosin (H&E), VEGF-A, CXCR4 and CXCL12 expression and compared with normal surrounding brain tissue. Thereafter the protein expression levels in VHL-related haemangioblastomas were compared to those in sporadic haemangioblastomas and correlated to the tumour and associated cyst size, and the VHL-gene and methylation status.
Currently VHL-mutation carriers undergo frequent surveillance for the detection of new VHL-related manifestations. The age to start and interval for surveillance is based on expert opinion. If estimations of the probabilities of developing VHL-related manifestations at various ages could be made, evidence-based decisions can be made on the age to start surveillance and surveillance intervals. In Chapter 6 we describe a retrospective analysis on the age of which a VHL-mutation carrier was detected with the first VHL-related manifestation. We analysed in 82 VHL-mutation carriers the cumulative proportion of carriers diagnosed with a manifestation during life. Using Poisson distribution models we calculated the time to detection of first manifestation and time to detection of subsequent manifestations. From these calculations we defined an age to start and interval for surveillance with a 5% detection probability.

VHL-related cerebellar haemangioblastomas are reported to oscillate between periods of growth and stability, but triggers for growth of these tumours are unknown. In addition, several case reports show that pregnancy or delivery in patients with VHL-disease can be complicated by progression of CNS haemangioblastoma or pheochromocytoma with consequences for maternal and neonatal outcome. Therefore, in Chapter 7 we studied in 29 VHL-mutation carriers the influence of pregnancy on the progression of VHL-related haemangioblastoma and the pregnancy related complication rate. We used medical charts and imaging reports from the VHL disease expertise centers in the Netherlands to retrospectively assess progression of haemangioblastomas before, during, and after pregnancy.

In Chapter 8 the main findings of this thesis are summarized and followed by a general discussion and future perspectives.
REFERENCES