Genetic contributions to the classification of renal cell cancer
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CHAPTER 1

1 GENERAL INTRODUCTION
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_Cancer, chromosomes and genes_

In limited time, a single fertilized egg gives rise to a complex multicellular organism consisting of differentiated cells arranged in a precise pattern. During embryonic development the different cell types become determined, each in its proper localization. The adult human body is a stable ecosystem in which one generation of cells succeeds another. A continuous process of cell proliferation is necessary throughout life to replace cells that have been lost due to injury or death, or to maintain populations of different cells that have short lifespans. In a normal situation there is a precise balance between cell proliferation and cell death.

A normal cell has multiple independent mechanisms to control growth and differentiation, encoded in genes in its DNA. Each DNA molecule is packaged in a separate chromosome, and the total genetic information stored in the chromosomes of an organism is said to constitute its genome. A typical human cell contains 46 chromosomes, consisting of 23 pairs, one member of which is inherited from each parent. Each gene is therefore present in twofold, except for X-linked genes in males.

Cancer is a caricature of developmental biology, caused by mutations in genes that control cell growth and differentiation. There are three classes of genes which, when altered by mutations, will contribute to the development of cancer. These are proto-oncogenes, tumor suppressor genes, and mutator genes. Normal or "wild-type" genes in all three categories affect the process of cell division or the fidelity of DNA replication. The products of proto-oncogenes act to stimulate normal cell proliferation. Oncogenes may arise from proto-oncogenes through a variety of events, including point mutations, multiplication (dose effect), and juxtaposition to other chromosome sequences. Oncogenes act in a dominant fashion. Tumor suppressor genes have a growth inhibiting function in the normal situation, and contribute to malignant growth through loss rather than activation. Their behavior is recessive, and both copies must be inactivated for tumor formation to occur. This inactivation may result from small deletions or point mutations, but also from loss of whole chromosomes. The so-called mutator genes are genes that in normal cells control the repair of DNA damage, maintain the fidelity of DNA synthesis and regulate proper cell division. Loss of function of these genes leads to a destabilization of the genome, thus facilitating the formation of additional genetic changes. Analogous to tumor suppressor genes, mutator genes behave in a recessive fashion, meaning that both gene copies have to be inactivated before destabilization occurs.

The development of cancer is a multistep process in which a single initially altered cell becomes malignant through a series of gradually progressive changes. Most cancers are clonal in origin, but tumor progression, i.e. acquisition of the capacity to invade and to metastasize, involves sequentially acquired genetic changes within the evolving neoplastic clone, leading to subpopulations with more aggressive growth characteristics. Therefore, despite their clonal origin, malignant tumors tend to be heterogeneous, comprising several genetic subpopulations of cells with different biological properties, regarding invasive and metastatic potential, and sensitivity to...
therapy. Chromosome aberrations in cancer cells can be divided into primary and secondary aberrations. Primary chromosome aberrations are directly related to tumorigenesis. They may occur as the sole abnormality and are often specifically associated with distinct tumor types. Secondary chromosome changes do not appear as the sole abnormality and act towards progression rather than tumor initiation. However, secondary changes do not occur randomly and their appearance may depend on the primary aberration and on the specific tumor type involved.

Cytogenetic analysis of human cancer cells has yielded a huge amount of information about the incidence and nature of chromosomal abnormalities in malignant cells [1] Specific chromosome changes have been identified in several types of cancer, and provide important tools in the diagnosis of these neoplasms. Notable examples in solid tumors are \( t(11;22) \) in synovial sarcomas, \( t(12;16) \) in mucoepidermoid carcinomas, and \( t(12;16) \) in myxoid liposarcomas. Molecular characterization of the loci involved in the specific translocations, has led to the isolation of several oncogenes, tumor suppressor genes, and tissue specific genes. Secondary changes, associated with tumor progression, can reveal information relevant for the prognosis.

Taken together, cytogenetic and molecular genetic analysis of tumor cells have, and will continue to provide essential information about the oncogenesis, progression, diagnosis, and prognosis of cancer.

Kidney development and function

The mature human kidney is a bean-shaped organ, which is on average 12 cm in length, 6 cm in width, and 2.5 cm in thickness [2]. It serves to convert over 1700 liters of blood per day into about 1 liter of a highly specialized concentrated fluid called urine [3]. In so doing, the kidney excretes the waste products of metabolism, precisely regulates the body's concentration of water and salt, maintains the appropriate acid balance of plasma, and serves as an endocrine organ, secreting such hormones as erythropoietin, renin, and prostaglandins. The physiologic mechanisms that the kidney has evolved to carry out these functions require a high degree of structural complexity.

Since the anatomy of the kidney has been detailed in several publications, only those features relevant to the understanding of renal cell cancer are emphasized [2]. Since renal cell cancer is commonly accepted to arise from cells of the mature renal tubular system, the structure of these functional units is explained. A mature uniferous tubule consists of a nephron (including a glomerulus and Bowman's capsule, a proximal convoluted tubule, loop of Henle, and distal convoluted tubule), and a collecting tubule. These two structures develop as separate entities from two different sources; the nephron develops from the metanephric mesoderm (metanephros), whereas the ureteric bud (mesonephros) is the primordium of the collecting tubule [4]. Mutual induction makes the ureteric bud grow and bifurcate and so form the collecting duct system, while the metanephric mesoderm is induced to move down the nephrogenic pathway [4]. When the distal part of a nephron contacts an arched collecting tubule, the two tubules become confluent and form a mature uniferous tubule (Figure 1). About 12 generations of nephrons form between the 8th and 34th week of gestation. By then kidney formation is completed and all renal growth from this point onward is the result of the enlargement of existing structures [2].
Renal cell cancer comprehends a heterogeneous group of tumors which account for 2% of all cancers diagnosed. They comprise 80-85% of all malignant kidney tumors and affect males twice as much as females. The overall incidence increases with each decade of life showing a peak in the sixth decade. In rare instances, RCC affects children and young adults [5-9]. No clear-cut geographical or ethnic preference has been reported for RCC, although the incidence is higher in Scandinavia and the United States as compared to Asia and Africa [10].

RCC is identified in patients with end-stage renal disease at a rate six times greater and at an age more than 10 years younger than in the general population [11]. A higher incidence of RCC has also been reported for patients with acquired cystic kidney disease, and tuberous sclerosis [12]. Environmental factors contributing to the development of RCC are smoking, particularly in men, and obesity, especially in women [13,14]. Occupational exposure to various hydrocarbons (gasoline, petroleum, and tar and pitch products), and asbestos also increases the risk on having this disease [15].

A lack of early warning signs is characteristic for RCC. Small, localized tumors rarely produce symptoms and therefore the diagnosis is often delayed until after the disease is advanced [3,16]. Classic diagnostic symptoms associated with RCC are hematuria (in 50 to 60% of patients), abdominal pain (in 40%), and a palpable mass in the flank or abdomen (in 30 to 40%). A combination of these three is found in only 10% of patients, and usually indicates advanced disease.
About 25-30% of patients present with metastatic disease at initial diagnosis, with lungs, bone, liver, and brain being the favorite sites. RCC is a highly unpredictable neoplasm with a tendency to recur or progress and cause death many years after initial treatment [17]. Pathologic stage is probably the single most important predictor of prognosis [5]. Although a TNM staging exists for RCC [18], the Robson system remains the most widely used [19]. Five year survival ranges from 65-85% for stage I patients, 45-80% for stage II, 15-35% for stage III, and 0-10% for stage IV patients. Among microscopic features, assessment of nuclear grade has the greatest prognostic significance [5,20]. In addition, Störkel and associates [21,22] developed a prognostic score based on the prognostic potential of the following parameters: TNM staging or Robsons staging, grading, cell type, growth pattern, and patient age. Although a correct prognosis of over 80% was achieved on the average for an individual case, this system has not been widely adapted.

Surgical resection is the treatment of choice for RCC. In case of localized disease, radical nephrectomy is usually curative. Partial nephrectomy has been attempted in patients with bilateral tumors and in patients with only one functional kidney. The overall survival of these patients has been found to be similar to that of patients with disease of similar stage who undergo radical nephrectomy. Therefore, nephron sparing surgery is presently also performed on patients with small lesions (<4cm) and a normal contralateral kidney [16]. Once the disease has spread, no adequate treatment is available, since hormonal and chemotherapeutic agents have little or no effect [16].

The classification debate

The classification of renal cell cancer has traditionally been based on the cytologic and architectural patterns of growth. The World Health Organization (WHO) nomenclature for kidney tumors includes benign and malignant tumors and is restricted to adenomas and carcinomas, the latter being divided into papillary and nonpapillary growth patterns. All other tumors are grouped under "others". In describing the architecture, the histological pattern (acinar, tubular, cystic, sarcomatoid, papillary, solid) and cytological features (clear, granular, oncocytic, pleomorphic/spindleshaped) are often included [10]. This classification does not allow extensive subtyping and gives little insight in the oncogenesis, tumor progression, and clinical behavior of RCC [23].

In 1986 Thoenes and Störkel [20] introduced a new, refined, classification for RCC, based on morphological, histochemical, and electron-microscopic data. A total of five basic subtypes of RCC are recognized, related to their origin from different parts and cells of the nephron (Figure 2). An extensive description of the morphological and genetic features of the different subtypes will be discussed in chapter 5. The cells of the proximal part of the renal tubule give rise to clear cell and chromophilic RCC, comprising 70-75% and 10-15% respectively. Chromophobe RCC (2-5%) and renal oncocytoma (4%) are derived from the intercalated cells of the collecting tubule, whereas Duct Bellini carcinomas (1%) find their origin in the principal cells of the medullary collecting duct. Variants can be assigned to most of these subtypes, resulting from an accumulation of mitochondria (eosinophilic variants). Sarcomatoid transformation may occur in any of the subtypes, except for the benign oncocytomas and represents an ultimate form of dedifferentiation. Sarcomatoid RCC usually is composed of sarcomatous and carcinomatous areas, and the diagnosis is made on the
properties of the carcinomatous component. When the entire neoplasm has a sarcomatous appearance, a correct diagnosis is difficult to make.

Three growth patterns are distinguished: solid, tubulo-papillary and cystic. Generally, one growth pattern predominates in a given tumor. A relation exists between cell type and growth pattern, but this is not an exclusive one. Clear cell and chromophobe RCC mainly have a solid growth pattern. Chromophilic RCC predominantly shows a tubulo-papillary architecture and renal oncocytoma is related to acinar growth. The Duct Bellini carcinomas are associated with both a compact and a tubulo-papillary growth pattern. Renal tumors are graded according to nuclear morphology, including size of nucleoli supplemented by cytoplasmic features, recognizing G1, G2, and G3/4 tumors. In 1995 this classification has been updated with the introduction of two new subtypes of RCC: neuroendocrine RCC and metanephroid renal adenomas [24]. These tumor types are rare entities and comprise less than 1% of RCC each. Since histological data concerning these subtypes is extremely scarce and no genetic data are available, these subtypes are excluded from the present study. Since the introduction of the classification of Thoenes and Störkel, their morphological subtyping has been validated by several cytogenetic and molecular genetic studies, showing distinct combinations of genetic changes present in each of the subtypes mentioned above. Hereditary and sporadic

Figure 2: Cytomorphological classification of renal-cell carcinomas (according to Thoenes and Störkel) in relation to the nephron and its cell types [20].
cases of clear cell RCC are characterized by deletions of the short arm of chromosome 3. Chromophilic/papillary tumors show a unique combination of autosomal gains: i.e. +7,+12,+16,+17, and/or +20. Chromophobe RCC is characterized by extensive non random chromosome losses, involving chromosomes 1, 2, 6, 10, 13, 17, 21, and the X or Y chromosome. Renal oncocytomas show a variety of chromosomal patterns, from which two genetically distinct subsets seem to emerge. One subset consistently shows the combined loss of chromosomes 1 and X/Y, whereas the other reveals a translocation involving breakpoint 11q13. In addition, both renal oncocytomas and chromophobe tumors exhibit changes in their mitochondrial DNA (mtDNA) and show telomere shortening and telomeric associations (tas). Up till now Duct Bellini carcinomas have been scarcely studied. Preliminary data indicate that loss of 8p and 13q may be important in their development.

**Aim of the thesis**

The histopathological classification of renal cell cancer is a constant matter of debate. This is not surprising, since renal cell cancer comprises a heterogeneous group of tumors, the phenotype of which may change dramatically during progression. On the other hand overwhelming evidence is available on the existence of genetically distinct subtypes of RCC. A specific combination of genetic changes marks each of the subtypes, whereas other changes have been related to progression. The generation of a genetic classification of RCC has been advocated by Kovacs and by us for several years [23,25-28]. The great advantage of such a genetic classification would be that genetic changes, heritable through cell division, are constant during tumor progression. Since genetic changes influence the morphology and biological behavior of tumor cells, there is a direct relation between both. However, the widely used WHO classification allows only subdivision into adenomas, carcinomas, and others. This classification does not do justice to the great diversity in genetic constitution, phenotypic appearance and biological behavior of renal neoplasms. The classification of Thoenes and Störkel, introduced in 1986, in which he different RCC subtypes are related to different cells and parts of the nephron, allows a more extended and refined subtyping, which may coincide with genetic subtyping. In addition the genetic constitution may lead to a refinement of the classification.

In order to elucidate whether, and to what extent the morphological classification of Thoenes and Störkel correlates with the observed genetic subsets of RCC, we combined the genetic data and morphological features of a large number of renal cell tumors, including own data and data extracted from the literature. In addition, we aimed to clarify possible pathogenetic relationships of different RCC subtypes and tried to elucidate the different oncogenetic steps important in the development and progression of these neoplasms, focussing mainly on the subtypes other than clear cell RCC. This survey is an extension of the data presented in the thesis of Dr. E. van den Berg-de Ruiter in 1993. The results of the present survey, as well as results from the literature, are compiled in a new oncogenetic model for renal cell cancer, depicted in chapter 5. The question is addressed whether the different genomic abnormalities found in the different subtypes of RCC may serve as tools for classification, and the identification of histogenetic relationships, and whether they will support, refine and/or change classification systems. The genomic alterations mark the location of
genes involved in the oncogenesis of renal cell tumors and may finally lead to their isolation and identification.