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Prognostic Factors in Renal Cell Carcinoma: 
An Evaluation of T-Stage, 
Histopathological Grade, p53, Ki-67, 
COX-2, and Her-2 Expressions

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1. Introduction

Kidney cancer\(^1\) represents 2-3% of all diagnosed malignancies worldwide although in some Northern and Central European countries the incidence is higher, even 4-5% (Ferlay, 2010). Kidney cancer is responsible for approximately 116,000 deaths per year worldwide (Ferlay, 2010). In the European Union (EU), the annual number of new kidney cancers was 73,171 in 2008 (Ferlay, 2010). The majority of renal cell carcinomas (RCCs) arise from the cells of renal proximal tubules of nephrons, but 5% of cases from the cells of the collecting ducts (Chao et al. 2002, Kovacs et al. 1997, Störkel et al. 1997) (Figure 1.). Renal tumors are members of a complex family with unique histology, cytogenetic defects and variable metastatic potential (Linehan et al. 2003, Thoenes et al. 1986). Of all RCCs, 70-80% is of conventional type, also known as clear cell RCCs. Of these, approximately 75% have a mutation in the von Hippel-Lindau tumor suppressor gene (VHL), in the short arm of chromosome 3 (Maxwell et al. 1999, Gnarra et al. 1994).

The annual increase in RCC incidence has been 2-4% since the 1970s (Finnish Cancer Registry 2007, American Cancer Society 2004, Mathew et al. 2002). This has been attributed to the use of radiological imaging which is able to find presymptomatic RCC lesions (Jayson and Sanders 1998), as well as the increased prevalence of etiologic risk factors, such as obesity (Chow et al. 2000) and cigarette smoking (Hunt et al. 2005). The increase has been highest in localized disease, especially in tumors with less than 4 cm in diameter (Hollingsworth et al. 2006). 30-60% of RCC tumors are found incidentally in abdominal imaging performed for some other reason than suspected renal tumor, such as the evaluation of non-specific musculoskeletal or abdominal complaints (Jayson and Sanders 1998). Macroscopic hematuria, palpable tumor and pain, together called the classic triad in RCC, indicate metastatic disease (Cunningham 1938). Metastatic disease is seen in 20-30% of RCC patients at diagnosis (Janzen et al. 2003, Mc

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\(^1\) In epidemiological statistics, RCC and renal pelvis cancer are usually not reported separately, but combined under the heading of kidney cancer (Parkin et al. 2003).
Nichols et al. 1981). Half of the patients diagnosed with local RCC will later have a recurrence of their cancer: two thirds within the first year (Janzen et al. 2003), and the majority within five years (Lam et al. 2005, McNichols et al. 1981). The risk for late recurrence, at over 10 years from nephrectomy, is at least 10% (McNichols et al. 1981).

![Diagram of renal cell carcinoma subtypes](image)

**Table 1.** The genetic changes that characterize the different RCC subtypes according to the Heidelberg classification (Modified from Bodmer et al. 2002).

<table>
<thead>
<tr>
<th>Conventional RCC</th>
<th>Papillary RCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3p</td>
<td>-Y</td>
</tr>
<tr>
<td>+5q</td>
<td>+3q</td>
</tr>
<tr>
<td>-6q</td>
<td>+7</td>
</tr>
<tr>
<td>-8q</td>
<td>+8</td>
</tr>
<tr>
<td>-9p</td>
<td>+12</td>
</tr>
<tr>
<td>-14q</td>
<td>+16, +17, +20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Collecting duct / tubule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromophobe RCC</td>
</tr>
<tr>
<td>-1q</td>
</tr>
<tr>
<td>-8p</td>
</tr>
<tr>
<td>-13q</td>
</tr>
<tr>
<td>-18p</td>
</tr>
</tbody>
</table>

The number reflects the chromosome in which its genetic aberration is located.
- means loss of function.
+ means gain of function.
p is the short arm of the chromosome.
q is the long arm of the chromosome.

Fig. 1. The genetic changes that characterize the different RCC subtypes according to the Heidelberg classification (Modified from Bodmer et al. 2002).
For those RCC patients with performance status enabling current treatments the expected five-year survival rate is slightly higher than 60% (Parkin et al. 2003). According to a few previous studies on long-term outcome for metastatic RCC (mRCC), the five-year survival is from 3% to 16% (Atzpodien et al. 2002, Motzer et al. 2000, Minasian et al. 1993) if metastasectomy has not been a possible treatment. For localized RCC, nephrectomy is the only curative treatment (Robson et al. 2002), and currently there is no adjuvant therapy in RCC. Possible treatments for mRCC, in addition to cytoreductive nephrectomy (Flanigan et al. 2001, Mickisch et al. 2001), are immunomodulators, such as interferon-α (IFN-α) (Kankuri et al. 2001, Pyrhönen et al. 1999), interleukin-2 (IL-2) (Négrier et al. 2007), and more recently tyrosine kinase inhibitors, such as sunitinib (Motzer et al. 2007), sorafenib (Escudier et al. 2007), and mTOR inhibitor temsirolimus (Hudes et al. 2007). Everolimus, another mTOR inhibitor, has an encouraging antitumor activity against mRCC (Motzer et al. 2008). The efficacy of bevacizumab, an antiangiogenesis monoclonal antibody, has also been shown when used with IFN-α (Bracarda et al. 2011, Rini et al. 2010, Yang et al. 2003). The Food and Drug Administration (FDA) and EU have also approved pazopanib, an angiogenesis inhibitor, with advanced RCC due to the efficacy of it in RCC (Sternberg et al. 2010). Ongoing clinical trials are addressing the role of targeted agents in adjuvant therapy in RCC (Choueiri et al. 2011). The efficacy of many potent novel targeted agents in RCC is under investigation in phase II and III trials, among these axitinib, a multitargeted tyrosine kinase receptor inhibitor (Goldstein et al. 2010), tivozanib, a pan-VEGFR tyrosine kinase inhibitor (De Luca and Normanno 2010), and ipilimumab, an anti-CTLA4 antibody (Yang et al. 2007). Additionally, vaccine therapy in RCC is being studied (Rini et al. 2011). The stabilization of the disease has been shown to be beneficial for the survival of mRCC patients (Thiam et al. 2010, Kankuri et al. 2001).

2. Staging and prognostic factors in RCC

2.1 Pathological tumor staging

In the 1960's, Robson et al, created the staging system based on physical characteristics and tumor spread with the addition of tumor venous invasion (Robson et al. 2002). The poor correlation between the different Robson stages and survival led to the recommendation to use the TNM (tumor, node, metastases) staging system. Since 1978, the TNM classification system for the extent of the tumor spread has integrated characteristics such as tumor size, vascular involvement, nodal spread and distant metastases (Bassil et al. 1985, Harmen 1978). pTNM classification system was updated by the Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC) in 1997 when the cut-off between T1 and T2 tumors was increased from 2.5 cm to 7 cm, in order to increase the difference in survival from these two tumor types. Analysis of outcome in nephrectomized patients showed that the 1997 TNM-system cut-off point between T1 and T2 tumors is too high, and a cut-off point of 4.5 – 5.0 cm has been suggested (Elmore et al. 2003, Zisman et al. 2001). In 2002, the pTNM classification system was revised: T1 was divided into T1a and T1b by a cut-off point of 4 cm, according to the suitability for partial nephrectomy, and prognostication (Sobin and Wittekind 2002, Guinan et al. 1997). A uniform staging classification, the TNM staging system, has improved the division of patients into radical or partial nephrectomy candidates. Additionally, it has increased the co-operation between oncologists and pathologists concerning the outcome of RCC patients (Janzén et al. 2003, Javidan et al. 1999). However, modifications in the TNM system may cause difficulty in comparing outcome data in different studies (Beldegrun et al. 1999, Störkel et al. 1989).
Pathological tumor stage (T-stage) has been observed to be the most important factor for locally confined RCC in predicting the survival of patients who have undergone nephrectomy (Kankuri et al. 2006, Delahunt et al. 2002). The observed five-year survival is approximately 75-80% for stage T1, 55% for T2, 40% for T3, and 20-30% for T4 (Sunela et al. 2009, Tsui et al. 2000). For patients with stage I disease (tumor confined to the kidney) the five-year survival is approximately 90%, and for those with stage I and histologic of chromophobe type it is almost 100% (Zisman et al. 2001). The five-year survival rate for stage III disease is approximately 50% (Zisman et al. 2001). There is an 80% difference in survival rates between patients with local disease compared to those with advanced disease and distant metastases (American Cancer Society 2004). In a retrospective review of 2 473 RCC patients from 1975 - 1985, regardless of T-stage, tumor size was observed to have an inverse association with survival (Guinan et al. 1995). In the study of Kankuri et al. (2006), in the analysis of those RCC patients who later developed metastatic disease, high T-stage caused twice the risk of metastatic disease and three times the risk of death compared with low T-stage which indicates that as the tumor size increases, the more aggressive its growth becomes and the more probable is tumor cell dissemination. T-stage is a prognostic factor for both metastases-free and overall survival in RCC patients.

T-stage can be used in estimating the correct duration and frequency of surveillance of RCC patients after nephrectomy. RCC with a diameter of less than 3.0 cm grows slowly; only 2.5% have metastases during the first three years (Bosniak et al. 1995). Therefore, in the treatment of those in whom surgery is contraindicated, careful monitoring (watchful waiting) by computed tomography (CT scan) may be used (Roberts et al. 2005, Bosniak et al. 1995). Previously, it has been suggested that T-stage is not an important prognostic factor in the survival of patients who have neither lymph node nor distant metastases (Giuliani et al. 1990). The therapeutic value of lymph node dissection remains unproven (Mickish 1999). T-stage alone has been pointed to be a valuable prognostic factor for survival, even when the status of lymph nodes is unknown (Kankuri et al. 2006). Additionally, a high T-stage has been used as an inclusion criterion for adjuvant treatments in trials (Atzpodien et al. 2005, Repmann et al. 2003).

Moreover, T-stage is an independent prognostic factor in mRCC patients (Kankuri-Tammilehto et al. 2010). In the study of Kankuri-Tammilehto et al. (2010) high T-stage caused twice the risk of death compared with low T-stage in mRCC. The association between T-stage and overall survival was also found in those with primary metastases at the time of nephrectomy (Kankuri et al. 2006). T-stage is not typically used in prognostic models in mRCC, a UCLA model (Zisman et al. 2002) being an exception. T-stage seems to be a good tool in prognostic evaluation in mRCC patients and could be included in prognostic models.

### 2.2 Histopathological tumor grading

In grading systems, the major criteria are nuclear and nucleolar appearances, while in some systems, tumor architecture and cell type is also included (Mostofi et al. 1998, Goldstein 1997, Fuhrman et al. 1982, Syrjänen and Hjelt 1978, Skinner et al. 1971). The WHO grading system is based on the size and prominence of nucleoli (Eble et al. 2004, Mostofi et al. 1998), while the Fuhrman grading system is based on nuclear size, shape, and presence or absence of nucleoli (Fuhrman et al. 1982). The WHO grading system contains three grades, whereas the Fuhrman contains four.
Several studies have failed to demonstrate any statistically significant differences in the survival of patients with different grades, when all three or four grades are analyzed separately (Kankuri et al. 2006, Rioux-Leclercq et al. 2000, Usbutyn et al. 1998, Selli et al. 1983) although when analyzing only the highest and the lowest grades the statistically significant difference in survival have been found (Kankuri et al. 2006). This is partly because, as yet, no consensus has been reached on a universal tumor grading system (Kanamaru et al. 2001, Medeiros et al. 1997). The observed five-year disease-specific survival (DSS) rate is approximately 90% for G1, 70-85% for G2, 45-60% for G3, and 15-30% for G4 (Gudbjartsson et al. 2005, Ficarra et al. 2001). Currently, different grading systems are utilized at different institutions. Tumor-grading systems have been criticized because of their subjectivity in tumor evaluations (Lanigan et al. 1994), and comparison of different patient cases with respect to histopathological grade is difficult. More quantitative measures which describe the size or the shape of the nuclei have been requested by pathologists. In 1997, an international consensus conference on RCC by UICC and AJCC outlined recommendations for the grading of RCC (Goldstein 1997): the grading system should be based on standardized and reproducible criteria that reflect the heterogeneity of nuclear and nucleolar features within a tumor, and each grade should result in significant differences in patient outcome. Recently again, a joint group of urologists and pathologists has published a proposal that the criteria for nuclear grading should be different for the different histopathologic subtypes of RCC according to the Heidelberg classification (Paner et al. 2006). Additionally, reducing the grades in the Fuhrman system has been proposed, for better outcome stratification (Rioux-Leclercq et al. 2007, Lohse et al. 2002, Bretheau et al. 1995). Overall, histopathological grade seem to be imprecise for prognostic evaluation in RCC patients (Uchida et al. 2002, Rioux-Leclercq et al. 2000, Lanigan et al. 1994).

2.3 Heidelberg and WHO classifications for typing of renal tumors

In Heidelberg, in October 1996, the morphology was combined with genetic findings for a new classification, called the Heidelberg classification of renal tumors, in a workshop organized by the UICC and the AJCC (Kovacs et al. 1997, Störkel et al. 1997). In addition to this, in 2004, WHO published the reassessed classification which is now based on both genetic and pathological abnormalities (Eble et al. 2004). Progress in our knowledge of genetic alterations leads to new suggestions for RCC entities (Eble 2003). With the progress of research, the Heidelberg classification may lead to more specific treatments in different subgroups of RCC patients. The 5-year DSS for locally confined RCC is for chromophobe RCC approximately 87-100%, for papillary RCC 87%, and for conventional RCC 70-75% (Cheville et al. 2003, Amin et al. 2002). In the case of sarcomatoid change, the survival decreases with the 5-year DSS of 35% (Amin et al. 2002). A very rare entity of collecting duct RCC is highly aggressive with highly decreased prognosis (Antonelli et al. 2003). The prognostic power of the Heidelberg classification has been investigated. The current Heidelberg classification does not have independent prognostic ability, and thus it should not be considered as a major prognostic variable comparable to T-stage and histopathological tumor grade (Patard et al. 2005). However, Heidelberg classification associates with metastases development, indicating that unclassified tumor type metastasizes with high probability (Kankuri et al. 2006). In future, with the progress of research, the Heidelberg classification may lead to more specific treatments in different subgroups of RCC patients (Störkel et al. 1997).
2.4 Prognostic models in RCC

The heterogeneity of RCC within the same T-stage and grade (Tsui et al. 2000) has resulted in a need for prognostic models for prognostication and treatment modality selection. Prognostic models, anagrams and nomograms, have been developed to find those nephrectomized RCC patients who potentially have a long-term recurrence-free interval and survival, as well as those mRCC patients who have long-term survival (Table 1.). The most often represented as an independent prognostic factors in metastatic RCC (mRCC) are performance status, time to metastases, number of metastatic sites, and prior nephrectomy. Therapies for mRCC cause a wide variety of adverse effects, which reduce the quality of life. Determining the prognostic factors for survival in mRCC patients is valuable in directing therapy for those patients who would benefit from it. Several models have been developed for predicting the likelihood of response to therapy and to predict survival. However, novel biomarkers are hoped to specify the diagnosis, staging, and prognosis and to guide targeted cancer therapies. Molecular tumor markers are expected to revolutionize the staging of RCC in the future (Srigley et al. 1997), as nowadays stratifying the patients into risk groups is largely done on the basis of clinopathological factors, e.g. clinical stage of the disease. Still, all the molecular mechanisms that affect the development, progression and clinical behavior of RCC are not known. Advances in the understanding of the pathogenesis, behavior, and molecular biology of RCC may help to better predict tumor prognosis, and thus improve survival of RCC carcinoma patients when a more tailored therapy can be given to each individual patient. Molecular biomarkers, such as p53, Ki-67 and COX-2, are candidates for defining prognostic subgroups (Delahunt et al. 2002), and for guiding targeted therapies (Masters 2007), as shown in the studies, where p53, Ki-67 and COX-2 had prognostic value in predicting survival. The following chapters describe in more detail about the value of them in the prognosis in RCC.

3. Biomarkers related to molecular mechanism in RCC

3.1 pVHL, von Hippel-Lindau protein, modulator of hypoxic response

pVHL, a tumor suppressor gene product, is expressed especially in the kidney’s proximal renal tubule (Corless et al. 1997, Iliopoulos et al. 1995). Approximately 61-75% of sporadic conventional RCCs contain mutations in VHL, in the short arm of chromosome 3 (3p25-26) (van Houwelingen et al. 2005, Maxwell et al. 1999, Gnarra et al. 1994), of which 50% show loss of heterozygosity (LOH) (Kovacs et al. 1997, Gnarra et al. 1994) and 10-20% silencing of the wild-type allele by promoter hypermethylation (Herman et al. 1994). VHL is associated with carcinogenesis. The function of pVHL is ubiquitylation of hypoxia-inducible factor (HIF); therefore, it modulates the hypoxic response; VHL protein can bind to hypoxia inducible factor-1 alpha (HIF-1α) and target this factor for destruction in the presence of oxygen. HIF in turn controls the expression of several proteins, including carbonic anhydrase 9 (CA9) and proteins involved in angiogenesis, i.e. vascular endothelial growth factor (VEGF) and EPO, via oxygen-dependent ubiquitination (van Houwelingen et al. 2005, George and Kaelin 2003). Normally, VHL down regulates vascular endothelial growth factor (VEGF) by different pathways. In VHL-defective cancer cells, increased concentrations of VEGF and EPO are observed.
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<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>No. of Patients</th>
<th>Therapy Administered</th>
<th>Tumor Subtype</th>
<th>Prognostic Factors</th>
<th>Prognostic Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motzer et al. (MSKCC)</td>
<td>2002</td>
<td>463</td>
<td>IFN-α</td>
<td>All</td>
<td>Performance status, time from diagnosis to start of therapy, LDH, hemoglobin, corrected calcium</td>
<td>Survival</td>
</tr>
<tr>
<td>Zisman et al. (UCLA)</td>
<td>2002</td>
<td>262</td>
<td>IL-2 or IFN-α (197 pts), other (65 pts)</td>
<td>All</td>
<td>T-stage, nodal involvement, nuclear grade, no. of symptoms, immunotherapy</td>
<td>Survival</td>
</tr>
<tr>
<td>Négrier et al. (Group Francais d’Immunotherapie)</td>
<td>2002</td>
<td>782</td>
<td>IFN-α ± IL-2</td>
<td>All</td>
<td>Performance status, no. of metastatic sites, disease-free interval, signs of inflammation, hemoglobin</td>
<td>Survival, rapid progression</td>
</tr>
<tr>
<td>Atzpodien (Medizinische Hochschule Hannover)</td>
<td>2003</td>
<td>425</td>
<td>IFN-α ± IL-2 ± 5-FU ± 13CRA</td>
<td>All</td>
<td>Neutrophil count, LDH, CRP, time from diagnosis to start of therapy, no. of metastatic sites, bone metastases</td>
<td>Survival</td>
</tr>
<tr>
<td>Motzer et al. (MSKCC)</td>
<td>2004</td>
<td>251</td>
<td>New agents</td>
<td>All, if cytokine refractory disease</td>
<td>Performance status, hemoglobin, corrected calcium</td>
<td>Survival for those who enter clinical trials of new agents</td>
</tr>
<tr>
<td>Choueiri et al. (Cleveland Clinic Foundation)</td>
<td>2007</td>
<td>358</td>
<td>IFN-α ± IL-2 ± chemotherapy</td>
<td>All</td>
<td>Performance status, hemoglobin, no. of metastatic sites, involved kidney of primary tumor</td>
<td>Long-term survival</td>
</tr>
<tr>
<td>Cho et al (Yonsei University)</td>
<td>2008</td>
<td>197</td>
<td>Immunotherapy</td>
<td>All</td>
<td>Performance status, N stage, no. of metastatic sites, sarcomatoid differentiation, liver metastasis</td>
<td>Survival</td>
</tr>
<tr>
<td>Motzer et al. (MSKCC)</td>
<td>2008</td>
<td>375</td>
<td>Sunitinib</td>
<td>Conventional RCC</td>
<td>Performance status, time from diagnosis to start of therapy, nephrectomy status, no. of metastatic sites, presence of liver or lung metastases, LDH, corrected calcium, hemoglobin, alkaline phosphatase, thrombosytosis</td>
<td>Probability of 12-month progression-free survival</td>
</tr>
</tbody>
</table>

LDH = lactate dehydrogenase  
MSKCC = Memorial Sloan Kettering Cancer Center  
UCLA = University of California  
Table 1. Prognostic algorithms and nomograms for survival in mRCC between 2000 and 2008.

3.2 CA9, hypoxia associated enzyme

CA9, a member of the carbonic anhydrase family, is suggested to play a role in the regulation of cell proliferation in response to hypoxic conditions. Low CA9 expression associates with the absence of VHL mutation and aggressive tumor characteristics in
conventional RCC (Pantuck et al. 2007). CA9 may indicate those patients who benefit from IL-2, as low CA9 expression associates with lower survival compared to high CA9 expression in mRCC patients who receive IL-2 (Atkins et al. 2005, Bui et al. 2003). It has also been suggested that CA9 may indicate those patients who benefit from CA9-targeted therapies. It is also being investigated whether CA9 may indicate those patients who are potential candidates for adjuvant therapy.

3.3 p53, biomarker of cell cycle point

p53, a tumor suppressor gene product, is a promoter of cell growth arrest and apoptosis (Choisy-Rossi and Yonish-Rouach 1998). Activated p53 elicits several cellular responses, including apoptosis and cell cycle arrest (Reich and Levine 1984), and responds to DNA damage at the restriction checkpoint of the G1 phase of the cell cycle (May and May 1999). In normal cells, p53 is usually undetectable (Finlay et al. 1988). Mutant p53 accumulates in cell nuclei and can be immunostained (Reich and Levine 1984), whereas wild-type p53, because of its short half-life, is usually undetectable by routine immunohistochemistry (Reich and Levine 1984). p53 accumulation and increased cell proliferative activity are parallel phenomena in RCC (Kankuri et al. 2006, Pinto et al. 2005). p53 may be upregulated in part by VHL, accounting for some of the tumor suppressive functions of VHL in RCC (Galban et al. 2003). p53 seems to associate weakly with tumor grade, as the association was seen only in univariate analysis. Nor was an association between p53 and grade observed in a previous microarray study (Zigeuner et al. 2004). In both studies, the nuclear grade was determined according to the WHO guidelines.

Published results on the association of p53 with survival have been controversial, some studies suggesting positive p53 associating with poor survival (Shvarts et al. 2005, Zigeuner et al. 2004, Uchida et al. 2002, Haitel et al. 2000), while others have observed no association (Itoi et al. 2004, Olumi et al. 2001, Rioux-Leclercq et al. 2000, Hofmockel et al. 1996). In the study of Phuoc et al. (2007), p53 was significantly associated with survival in univariate analysis, but the association was not independent. In a tissue array study on metastasized patients, overexpression of p53 was associated with impaired DSS in renal carcinoma (Kim et al. 2004). In some studies, the association of p53 and survival has been investigated in a group of RCC patients with both locally confined and primary metastatic RCC; thus, patient selection varies in different studies (Olumi et al. 2001). The study of Kankuri et al. (2006) indicates that p53 is not able to predict which patients will develop metastatic disease after nephrectomy, but interestingly, they predict poor survival in mRCC patients (Figure 2.). Therefore, p53 can help in determining metastatic patients with a poor prognosis and, e.g., those who might benefit from aggressive treatment, such as high-dose interleukin-2 (Spanknebel et al. 2005) or temsirolimus (Hudes et al. 2007).

3.4 Ki-67, proliferation marker

Ki-67, a proliferation biomarker, is expressed throughout the active phases of the cell cycle, and serves as a good marker for proliferative activity in cell nuclei (Gerdes et al. 1984). Ki-67 accumulates during the cell cycle from G1 to mitosis, and is at its lowest level after mitosis (du Manoir et al. 1991). The percentage of nuclei staining by immunohistochemistry reflects Ki-67 expression (Olumi et al. 2001). An association between Ki-67 and high T-stage and metastases development have been observed
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(Kankuri et al. 2006, Dudderidge et al. 2005, Rioux-Leclercq et al. 2000), indicating that Ki-67 is a marker for aggressive disease in RCC with an increased risk of early metastases development. Ki-67 has been reported to independently predict survival following nephrectomy in many studies (Dudderidge et al. 2005, Bui et al. 2004, Itoi et al. 2004, Rioux-Leclercq et al. 2000, Aaltomaa et al. 1997). Ki-67 has been observed to increase in sarcomatoid change (Kanamaru et al. 1999), indicating different protein expression profiles in different entities according to the Heidelberg classification.

Fig. 2. Kaplan-Meier survival curve for p53 in mRCC (n=66) (Kankuri et al. 2006).

Dudderidge et al. (2005) found Ki-67 to be an independent prognostic factor for disease-free survival in nephrectomized RCC, but opposite results have also been published (Donskov et al. 2004, Kim et al. 2004, Yildiz et al. 2004). No association between Ki-67 alone and survival in locally confined RCC patients was found in the study of Kankuri et al. (2006). The differences in the classification of metastases are seen: Kim and coworkers (2004) classified both distant and local lymph node metastases as metastatic disease, whereas in the study of Kankuri et al. (2006), only tumors with distant metastases were classified as metastatic. However, Ki-67 predicts poor survival in mRCC patients (Figure 3.). Therefore, in addition to p53, Ki-67 can help in determining metastatic patients with a poor prognosis and, e.g. those who might benefit from aggressive treatment.
Fig. 3. Kaplan-Meier survival curve for Ki-67 in mRCC (n=66) (Kankuri et al. 2006).

### 3.5 COX-2, biomarker for inflammation and neoplasia


Cytoplasmic/membranous COX-2 staining by immunohistochemistry reflects COX-2 protein expression (Cho et al. 2005). The study results on associations of COX-2 with tumor stage, grade, and survival have been contradictory. Yoshimura et al. (2004) demonstrated that COX-2 was expressed at its highest in G1, as well as in pT1 RCC tumors, compared to other RCC...
tumors in grade and T-stage, while in Hashimoto et al’s study (2004), more COX-2 was found at the higher tumor grade, as well as stage. Kankuri-Tammilehto et al. (2010) found no association between COX-2 and tumor grade or T-stage. A significant association has been observed between COX-2 and Ki-67 expression in the study of Miyata et al. (2003), whereas Kankuri-Tammilehto et al. (2010) found no association between them. No association between COX-2 and p53 has been found in studies (Kankuri-Tammilehto et al. 2010, Cho et al. 2005).

Kankuri-Tammilehto et al. (2010) found that the proportion of COX-2 positive tumors is highest in RCC with the ability to develop later metastases, when compared to both RCC without metastatic potential and RCC with primary metastases. This finding was new. Previously, Miyata et al. (2003) observed that positive COX-2 expression associated with primary metastases in univariate analysis (when M0-patients were compared to M1-patients). Cho et al. (2005) found no association between positive COX-2 expression and metastases (when M0-patients were compared to M1-patients, or appearance of metastatic disease was compared to non-metastatic disease). In those studies, the method of analysis differs from that of the study of Kankuri-Tammilehto et al. (2010), where patients were divided into three categories according to the appearance of metastases. According to the study of Kankuri-Tammilehto et al. (2010), metastases-free survival is longer in patients with COX-2 positive tumors. The median metastases-free survival was 46 months in RCC with COX-2 positivity compared to 15 months in RCC with COX-2 negativity (Figure 4.).

Fig. 4. The prognostic value of COX-2 for metastases-free survival from nephrectomy in RCC patients who later developed metastatic disease (n=32, Kaplan-Meier method): the median metastases-free survival time was 46 months with COX-2 positivity, and 15 months with COX-2 negativity (Kankuri-Tammilehto et al. 2010).
results indicate that COX-2 positivity associates with the delay of metastatic formation in RCC patients who do not have disseminated disease at presentation, and that COX-2 negativity associates with an aggressive phenotype in mRCC disease.

Few studies have reported the results of an association between COX-2 expression and survival in RCC patients. Previously, Miyata et al. (2003) found that the five-year survival of patients with COX-2 positive tumors from nephrectomy was 66%, and of COX-2 negative patients 91% (Miyata et al. 2003). In Miyata’s study, the patients were 86% M0 and 14% M1 at nephrectomy. Previously, no results of COX-2 and overall survival in mRCC patients have been published. The study of Kankuri-Tammilehto et al. (2010) indicates that COX-2 positivity predicts improved overall survival in patients with mRCC treated with IFN-α. This is in line with the previous study of Rini et al. (2006), in which COX-2 positivity associated with longer time to progression in the patients treated with celecoxib plus interferon-α. Kankuri-Tammilehto et al. (2010) observed no association between COX-2 staining and response to IFN-α alone, while Rini et al. (2006) reported that all the RCC patients with objective responses to celecoxib plus interferon-α expressed COX-2 staining. Additionally, COX-2 does not associate with the Heidelberg classification (Kankuri-Tammilehto et al. 2010, Yoshimura et al. 2004).

3.6 Her-2, biomarker of proto-oncogene product

Her-2, a proto-oncogene product, is a member of the ErbB family of receptor tyrosine kinases. Her-2 functions in secretory epithelial tissues, and regulates intracellular signaling cascades (Arteaga et al. 2001, Olayioye et al. 2000). Her-2 is over-expressed in approximately 20-30% of human adenocarcinomas (Latif et al. 2002, Lipponen et al. 1994, Slamon et al. 1989), and the over-expression is associated with metastatic phenotype and poorer prognosis, e.g. in breast and ovarian cancer (Slamon et al. 1989).

Gene amplification of Her-2 can be investigated by cytogenetic analyses, such as fluorescent in situ hybridization (FISH), chromogenic in situ hybridization (CISH), and polymerase chain reaction (PCR). In breast cancer, FISH and CISH positivity are accurate predictors of response to trastuzumab (anti-Her2 therapy) (Isola et al. 2004, Lebeau et al. 2001). Receptor-mediated targeted therapy with Herceptin® (RhuMAb HER-2), a recombinant humanized monoclonal anti-Her-2 antibody, has improved the survival of breast carcinoma patients both in adjuvant therapy and in therapy for metastatic disease (Smith et al. 2007, Montemurro et al. 2003).

Membranous staining of HER-2 in immunohistochemistry reflects HER-2 protein expression (Zhang et al. 1997). Her-2 receptor-specific tumor toxin, in an animal model, effectively reduced pulmonary tumors of advanced RCC (Maurer-Gebhard et al. 1998). Parallel associations of Her-2 expression between tumor stage and grade in RCC patients have been observed in many studies (Zhang et al. 1997, Stumm et al. 1996), although in the study of Seliger et al. (2000) no such association was found. In the study of Hofmockel et al. (1997), higher tumor grades were seen when Her-2 expression was low, and higher T-stage associated with high Her-2. In the study of Phuoc et al. (2007), Her-2 protein expression did not correlate with Ki-67 protein expression.

In most HER-2 gene amplification studies, Her-2 gene amplification was observed neither by FISH analysis (Latif et al. 2002), messenger ribonucleic acid (mRNA) analysis (Stumm et al. 2002),
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1996), nor PCR analysis (Selli et al. 1997, Zhang et al. 1997). Selli et al. (1997) found HER-2 gene amplification in collecting duct RCC cases (45%). Therefore, HER-2 gene amplification may be more pronounced in collecting duct RCC, than in other more common RCC types (Matei et al. 2005, Zhang et al. 1997). The association of HER-2 gene amplification and HER-2 protein expression with the prognosis of RCC patients has been estimated in few studies and the results have been contradictory (Phuoc et al. 2007, Lipponen et al. 1994). Further studies are needed to determine whether HER-2 protein expression or HER-2 gene amplification may be used as prognostic factors in RCC patients.

3.7 Incidence of p53, Ki-67, and COX-2 expressions

The incidence of p53- and Ki-67-positive expression in RCC tumors was low in RCC studies (Kankuri-Tammilehto et al. 2010, Kirkali et al. 2001, Haitel et al. 2000, Rioux-Leclercq et al. 2000). It is known that in addition to melanoma, RCC belongs to tumors with a low incidence of p53 mutations compared to, e.g. prostate and bladder cancer (Haitel et al. 2000, Kirkali et al. 2001, Rioux-Leclercq et al. 2000). The low p53 mutation in different cancers (Olivier et al. 2002) and the low immunohistochemical staining of RCC tissue blocks for the p53 protein in studies (Haitel et al. 2000, Rioux-Leclercq et al. 2000) suggest that mutations in p53 result in an accumulation of the p53 protein. In the study of Oda et al. (1995), p53 expression was found only in those components with p53 mutations, mainly in the sarcomatoid components. The 10% cut-off value of p53 and Ki-67 was often selected to achieve statistically reliable results, and in accordance with previous studies on the subject (Kankuri et al. 2006, Olumi et al. 2001). Previously published reports indicate that the proportion of COX-2 positive cells varies in human RCCs (Cho et al. 2005, Miyata et al. 2003). In the study of Kankuri-Tammilehto et al. (2010), weak intensity of COX-2 staining was considered as COX-2 negative, which resulted in a lower number of positive COX-2 cells than in some other RCC studies (Tuna et al. 2004, Cho et al. 2005). For comparison, in the study of Miyata et al. (2003), the criterion for positive COX-2 expression was 5%, whereas in the study of Kankuri-Tammilehto et al. (2010), it was considered to be 10%. Also different antibodies have been used in other studies (Rini et al. 2006, Cho et al. 2005, Hashimoto et al. 2004). This fact and the criteria for immunohistochemical classification may contribute to the difference in the results. Validation of immunohistochemical methods is needed before the methods could be widely adopted for in clinical use.

3.8 Combining markers

In multivariate analysis, COX-2 and Ki-67 were independent variables, indicating that they are both stronger biomarkers than p53 for the development of metastases in RCC. However, combining markers may specify prognostic subgroups better than observing a single marker. As shown in a study by Haitel et al. (2000), p53 was not an independent predictor for survival, but p53 and mdm2, a negative regulator of p53, showed a strong association with poor survival. In the study of Kankuri et al. (2006), in RCC patients, double positivity for p53 and Ki-67 expression seems to indicate a higher probability of metastases than either marker alone. Additionally, combining COX-2 and Ki-67 increases their ability to predict survival in mRCC (Figure 5.). In this study, median overall survival time of RCC with COX-2 negativity/Ki-67 positivity was 19 months, which was almost five times shorter than of RCC with COX-2 positivity/Ki-67 negativity. Median overall survival time of RCC with
COX-2 negativity alone was 28 months, which was three times shorter than that of RCC with COX-2 positivity.

Fig. 5. The prognostic value of covariation of COX-2/Ki-67 for overall survival from nephrectomy in RCC patients with metastases (either primary presentation or later) (n=57, Kaplan-Meier method): the median overall survival time was 97 months with COX-2 positivity/Ki-67 negativity, and 19 months with COX-2 negativity/Ki-67 positivity (p=0.004) (Kankuri-Tammilehto et al. 2010).

Prognostic markers can be used in patient counseling, to select treatment modalities, and to determine eligibility for clinical trials. Different prognostic models have been created to specify the prognosis of RCC patients; they typically include conventional prognostic markers. However, combining biomarkers and conventional clinical markers seems to predict DSS more accurately than grade or TNM stage alone, both in locally confined and metastatic RCC (Kim et al. 2004).

3.9 Trends in the use of biomarkers

Prospective clinical trials on the clinical use of p53, Ki-67, and COX-2 protein expression in predicting overall survival could answer the question of whether the expression of these biomarkers can be reliably used in mRCC. These biomarkers cannot predict response to IFN-α (Kankuri-Tammilehto et al. 2010). Whether these biomarkers can predict response to novel targeted therapies should be investigated in trials. The new era of genetic cancer studies shows great promise in terms of patient evaluation for new targeted therapies or immunotherapy. By means of the tissue microarray technique, thousands of tumors can be
investigated simultaneously to determine the protein expression profile. However, creating a consensus in the tissue microarray construction protocol is challenging, as RCC is a relatively large-size tumor of a highly heterogeneous nature (Signoretti et al. 2008). At current, whole tissue sections are considered the gold standard, but the more cores per tumor are sampled the fewer errors are introduced by limited sampling. Using gene chips to profile kidney tumors defines the genes that determine patient survival and response to therapy, thus enabling precise prognosis determination and individual treatment planning (Tan et al. 2008). Additionally, tissue microarrays enable the analysis of protein expression profiles in specimens to determine their potential clinical significance and role in RCC biology.

4. Conclusion

RCC is an extremely heterogeneous disease, with patients having an overall survival from a few months to several years. For those RCC patients with performance status enabling current treatments, such as nephrectomy, immunomodulators, and more recently targeted therapies, the expected five-year survival rate has been slightly higher than 60%. Metastatic disease is seen in 20-30% of RCC patients at diagnosis. The five-year survival for metastatic RCC is from 3% to 16% if metastasectomy has not been a possible treatment. Currently, tumor (T)-stage is the best known prognostic factor for locally confined RCC. T-stage is a prognostic factor for both metastases-free and overall survival in locally confined RCC patients as well as in overall survival in metastatic RCC (mRCC). No consensus has been reached on a universal histopathologic tumor grading system. Several published reports have pointed out the differences in survival between the highest and the lowest tumor grades, even though when all three or four tumor grades were analyzed separately, the differences were no longer statistically significant. The heterogeneity of RCC within the same T-stage and grade has resulted in a need for more specific prognostic markers, related to molecular mechanisms of RCC, to specify diagnosis, staging and prognosis. Prognostic markers can also be used in to select treatment modalities, help in surveillance, and to determine eligibility for clinical trials. p53 associates weakly with tumor grade whereas Ki-67 associates with T-stage and metastatic development, indicating that Ki-67 is a marker for aggressive disease in RCC with an increased risk of early metastases development. The proportion of COX-2 positive tumors is highest in RCC with the ability to develop later metastases, when compared to both RCC without metastatic potential, and RCC with primary metastases. Metastases-free survival is longer in patients with COX-2 positive tumors compared to COX-2 negative tumors. These data show that COX-2 negativity associates with an aggressive phenotype in mRCC disease. COX-2 and Ki-67 alone are stronger biomarkers than p53 for the development of metastases in RCC. Her-2 seems to associate with p53 and Ki-67, but results of associations between Her-2 and survival have been contradictory. Few studies have been published on the significance of Her-2 protein expression or Her-2 gene amplification in RCC, so more studies are warranted. p53 or Ki-67 alone are not valuable prognostic markers in locally confined RCC, but they can predict poor survival in mRCC. Therefore, p53 and Ki-67 can help in determining metastatic patients with a poor prognosis and, e.g. those who would benefit from high-dose IL-2 or temsirolimus. COX-2 positivity predicts improved overall survival in patients with mRCC treated with IFN-α. p53, Ki-67, and COX-2 cannot predict response to IFN-α. Investigating the ability of p53, Ki-67, and COX-2 protein expression to predict overall survival in a
prospective clinical trial would answer the question of whether these biomarkers can be reliably used in mRCC. Combining the results of COX-2 and Ki-67 expression, may predict overall survival in mRCC. In predicting the development of metastases in nephrectomized RCC patients, COX-2 alone or a covariation of p53 and Ki-67 seem to have prognostic value. Combining p53 or COX-2 with Ki-67 may result in more specific prognosis staging in RCC than observing a single marker. In future, using the tissue microarray technique, the protein expression profile with several biomarkers can be determined quickly. Further investigations are needed on the reproducibility of staining of these novel biomarkers, and on validation of the ability of the expressions to discriminate clinical outcome in RCC. Findings on novel biomarkers have increased our understanding of the molecular biology of locally confined RCC patients and metastatic RCC patients. RCC is characterized by high resistance to radiation and chemotherapy, which may be due to the suppression of apoptotic mechanisms, such as the p53 tumour suppressor pathway. In RCC patients, Ki-67 expression is not very high, which may partly explain RCC’s resistance to chemotherapy. Specification of the roles of novel tumor-related molecular prognostic factors might be translated into prognostic tools that could be used in clinical work.

5. References


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Zisman A et al. (2001). Re-evaluation of the 1997 TNM classification for RCC: T1 and T2 cutoff point at 4.5 cm rather than 7 cm better correlates with clinical outcome. J Urol 166: 54-58.
The field of renal cell cancer has undergone a significant resurgence. This book summarizes up-to-date research and innovative ideas for the future in this rapidly changing field, which encompasses medicine, surgery, radiation oncology, basic science, pathology, radiology, and supportive care. This book is aimed at the clinician or scientist who has an interest in renal cell cancer, whether they are academic or nonacademic. The book covers tumor biology, molecular biology, surgery techniques, radiation therapy, personal testimonies, and present and future treatments of the disease that are on the horizon. The goal was to produce a textbook that would act as an authoritative source for scientists and clinicians and interpret the field for trainees in surgery, medicine, radiation oncology, and pathology.

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