Renal cell carcinoma: Resistance to therapy, role of apoptosis, and the prognostic and therapeutic target potential of TRAF proteins

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ARTICLE INFO

Article history:
Received 7 July 2011
Accepted 13 November 2011

ABSTRACT

Renal cell carcinoma (RCC) is the commonest of the renal neoplasms. Although surgery and cryoablation are successful curative treatments for localized RCC, most patients are diagnosed with advanced or metastatic RCC, which has a poor prognosis. RCC are a heterogeneous set of cancers that have traditionally been classified and staged using cellular characteristics, size, local extension and distant metastases. Current staging systems provide good prognostic information, but it is very likely that the identification of new more accurate and predictive prognostic markers, not currently included in traditional staging systems, will improve the outcome for RCC patients. For this reason, increased knowledge of the underlying molecular characteristics of RCC development and progression is necessary. In most cancers, but especially RCC, deregulated control of apoptosis contributes to cancer growth by aberrantly extending cell viability and facilitating resistance to cancer therapies. Here we present the hypothesis that select members of the tumor necrosis factor (TNF) superfamily, the TNF receptor-associated factors (TRAFs), have a role in RCC apoptosis and may have prognostic significance for RCC. Candidate biomarkers for RCC are few, and the TRAFs may be important inclusions in panels of biomarkers for RCC. TRAFs may also be potential molecular targets for new therapies, either through their ability to promote apoptosis in the cancers themselves, or through their ability to modulate the immune defence against cancer progression. Some support data are presented here for our hypothesis. However, these novel concepts need further careful analysis to allow clinicians and oncologists any assistance for earlier detection of RCC and for characterizing patients with RCC for individualised targeted therapy.

Introduction

Renal neoplasms are not insignificant cancers, being the tenth leading cause of cancer mortality in Western industrialized countries and approximately 3% of the total human cancers. They are also increasing in incidence, by 1.5–5.9%, on an annual basis [1–3]. Since 1950, the accumulated increase is 126%. Ultrasound, computed tomography, magnetic resonance imaging, intravenous urography and angiography are widely used for general disease diagnosis, with approximately 80% of people having renal neoplasms diagnosed as an asymptomatic incidental finding of these investigations [4]. Thus, although the reported increase in renal neoplasms may, in fact, be related to improved techniques that diagnose cancers or other general disease located spatially near the kidney, there also appears to be a real increase in the incidence and prevalence of renal neoplasms [3]. Clinically-localised renal cancers can be successfully excised surgically or treated with cryoablation, with complete cure possible, however metastasis is frequent, and may have already occurred even with early diagnosis [5]. The ability of the kidney to compensate functionally when partly destroyed means that early loss of renal function, caused in part of the kidney by the growing renal mass, is often not detected [6,7]. Renal cell carcinomas (RCC) make up approximately 90% of the renal neoplasms [8]. Most epidemiological studies do not differentiate between clinically-localised renal masses and metastatic RCC, but it is likely that the incidence of malignancies localised to the kidney and metastatic RCC have increased correspondingly. Metastatic RCC are difficult to remove surgically, and are highly resistant to conventional cancer treatments, such as radio-, immuno- and chemo-therapy [7]. Discovery of the molecular determinants of this resistance to therapy in RCC has proved to be elusive.
Interference with the process of apoptosis, or programmed cell death, may occur at many different points in the cell death process, including mutation or loss of key genes and their transcripts, synthesis of splice variants of genes, post-translational events, the appearance or disappearance of inhibitors, and protein degradation [9]. One of the least investigated apoptosis pathways in resistance of RCC to therapies, but having success as a molecular marker or target for cancer therapy in other malignancies, is the tumor necrosis factor (TNF) family of proteins. In myeloid tumors, TNF is required for the induction of suppressor cells that inhibit tumor growth. In squamous cell carcinomas, lung cancer, and ovarian cancer, TNF promotes tumor growth through its induction of an inflammatory response that inhibits cell-mediated immunity (squamous cell carcinomas), enhances metastases by inducing S100A8/A9 which recruits tumor cells (lung cancer), and small trials of TNF inhibitors in humans have resulted in cancer stabilization (ovarian cancer) [10]. TNF-α is normally considered a cell death ligand and so it is likely that its effect is often on the immune cells that would normally detect and delete tumor cells. In the extrinsic cell death pathway, death ligands like TNF-α and TNF-related apoptosis-inducing ligand (TRAIL) interact with their respective death receptors TNF-receptor (TNFR), and the TRAIL receptor (TRAILR) [11,12]. The binding of the ligand to the receptor activates the receptor and stimulates recruitment of adaptor proteins. These form a complex called the death-inducing signaling complex and there is subsequent activation, or cleavage, of the initiator caspases to start a "caspase cascade" to a cell death outcome.

**TNF receptor-associated factors (TRAFs)**

TNFR-associated factors (TRAFs) have also demonstrated altered expression in development of some non-RCC cancers [13,14], are a potential target for treatment of these cancers [15], and the same may be found for RCC [16]. TRAFs are primarily involved in the regulation of inflammation, antiviral responses and apoptosis [17]. They link the TNFR subfamily to signaling cascades (Fig. 1) [18]. TNF acts via its receptors, TNFR1 and TNFR2, to activate the TRAF pathways. The outcome of activation of these pathways in cell death or cell survival and proliferation is generally unknown, but death signaling pathways (c-Jun N-terminal kinase/JNK, apoptosis inhibitor/API, receptor interacting protein/RIP and p38-mitogen activated protein kinase/p38MAPK) and survival signaling pathways (for example, nuclear factor-kB/NF-kB) have been identified. Activation of the death domains (DD) like TRADD (TNFR activated DD) and FADD (Fas ligand activated DD) also activates death pathways, like the caspase-8/caspase-3/apoptosis pathway, and it likely there is interaction between TRADD and FADD [18]. TRAF proteins are expressed in normal and diseased tissue in a regulated fashion, implicating a role in regulating physiological and pathological processes [19]. Despite their recognized importance in these processes, little is known about the TRAFs in RCC. One constant problem for analyzing function of any gene or protein in development and treatment of RCC is the sheer heterogeneity of this cancer, with its many distinct histological subtypes. A summary of the subtypes of RCC will now be given to exemplify the heterogeneity of this cancer, before summarizing some data on apoptosis in RCC resistance to therapy and finally presenting data in support of the hypothesis of this paper.

**Classification of renal cell carcinoma**

RCC are solid tumors usually arising from the epithelial cells of the renal tubule, most commonly the proximal tubular epithelium [20] although other parts of the nephron epithelium may also...
provide the transformed cells. One of the most comprehensive clas-
sifications [21] lists RCC as clear cell (ccRCC), papillary and
sarcomatoid, chromophobe, collecting duct, medullary mucinous
and spindle cell carcinoma, RCC-associated Xp11.2
translocations/TFE3 gene fusion, and carcinoma associated with
neuroblastoma. The most common form, ccRCC, accounts for
approximately 70% of RCC [21]. These tumors are typically cortical
in locality and golden yellow due to abundant cholesterol, neutral
lipids and phospholipids especially in the cytoplasm of the cancer
cells. They are solitary tumors, randomly distributed with equal fre-
quency in either kidney and often protruding from the renal cortex
as a rounded mass with a pseudocapsule, with the adjacent “nor-
mal” kidney being well-demarcated. Histologically, the cytoplasm
appears very pale or clear. ccRCC are considered to originate from
the renal proximal tubular epithelium [20]. Metastasis of ccRCC is
often haematogenous via the vena cava, primarily to the lung,
although bone, brain and lymphatic metastases also occur. The
majority of sporadic ccRCC has a loss of chromosome 3p and inac-
tivating mutations of the Von Hippel Lindau (VHL) gene [22–24].
The VHL protein is linked to regulation of hypoxia-inducible factor
(HIF) and vascular endothelial growth factor (VEGF) and the major-
ty of treatment trials for ccRCC now target the HIF/VEGF pathways
involved in angiogenesis [24].

Of the other subtypes of RCC, papillary RCC are the second most
common (10–15%). The name “papillary” is derived from the
development of papillary or tubulo-papillary tumor architecture in
the renal parenchyma [25]. These RCC are sub-classified into
Type I which are associated with the activating mutation of the
c-Met oncogene, and Type II which are associated with the muta-
tions in the fumarate hydratase gene and often of a higher nuclear
grade than Type I. Papillary RCC originate from the proximal tubu-
lar epithelium and have increased incidence in patients with end-
stage renal disease [26,27]. Chromophobe RCC (5%) have large pale
cells, but the cells are eosinophilic, have a peri-nuclear halo,
and usually form solid sheets with concentration of the largest
cells around the blood vessels. If inherited, chromophobe RCC
derive from a syndrome called Birt Hogg Dube [28]. These RCC
rarely metastasise, and then only late in their clinical course. Col-
lecting duct RCC are a rare but aggressive variant of kidney cancer
[29]. Most are located centrally in the medullary zone and have
associated tubulo-epithelial dysplasia or atypia. They often display
high grade (Fuhrman Grade 3 and 4) nuclear features [21]. Approx-
imately 5% of RCC are “unclassified” because their appearance does
not fit any of the other categories [30,31]. They are often tumors
with varied appearances and genetic lesions [30]. Sarcomatoid
RCC are an aggressive development in RCC, with sheets of malig-
nant spindle cells that have features of both stromal and epithelial
cells. Sarcomatoid differentiation can be identified in any subtype
of RCC, but it is typically seen in papillary RCC [25,32–34]. Sarco-
matoid components increase patient risk of progression of RCC
[33]. In a separate classification from RCC, renal oncocytoma is a
benign renal epithelial neoplasm. Its origins may have some link
with RCC, and it is sometimes mistaken for chromophobe RCC,
and so it is mentioned here [21,35]. These benign growths are usu-
ally a well-circumscribed, homogeneous, mahogany-brown mass
with a characteristic central stellate scar (up to 33% of the cases).

Molecular targets for renal cell carcinoma

As for any cancer, modern treatments for RCC are now aimed at
the molecular targets that are involved in their development. The
abnormal expression of oncogenes, inactivation of tumor suppres-
sor genes, and random variations in gene expression, at least in
part, provoke the transformed cancer cell into uncontrollable
growth. Various mechanisms by which cancer cells become
resistant to treatment are also important in RCC progression. Al-
tered cell cycle checkpoint genes, faulty DNA repair genes and drug
efflux pumps, and increased or altered drug targets all play a role
[6]. The heterogeneity within different RCC subtypes means there
are different patterns of molecular defects within the tumor cells,
including molecules that control cell proliferation, cell death, cell
motility, and angiogenesis supplying the growing cancer with oxy-
gen and nutrients. These defects affect tumor growth but may also
affect tumor response to therapy. RCC in general often have PTEN
(phosphatase and tensin homolog deleted on chromosome ten) tu-
mor suppressor gene mutations [36]. The mutations activate the
phosphatidylinositol 3-kinase (PI3K)/Akt pathway, contributing to
细胞 proliferation and differentiation in RCC. Mutated PTEN
may also allow accumulation of p21, a cell cycle regulator that is
known to act in resistance to chemotherapies. ccRCC and papillary
RCC have activated PI3K/Akt more often than chromophobe or sar-
comatoid RCC, and increased cytoplasmic and nuclear phospho-
PI3K/Akt levels may be independent prognostic factors for dimin-
ished patient survival [37]. However, there are many gene modifi-
cations in RCC that may determine their persistent growth and
resistance to treatments [38,39].

Another of the more general molecular defects in RCC involves
multi-drug resistance (MDR). The MDR1 gene acts in normal tis-
sues by exporting unnecessary or toxic exogenous substances or
metabolites out of the body [40]. MDR1 is expressed normally in
the renal proximal tubular epithelial cells from which most RCC
are thought to arise. Its relatively high expression is retained with
malignant transformation, hence controlling membrane fluxes,
including the passage of drugs toxic to the cancer cells across the
cell membrane [41]. Although MDR is undoubtedly important in
RCC development, progression and treatment, it does not represent
the complete reason for resistance of RCC to therapy. Defining
velar molecular mechanisms and pathways of RCC resistance to ther-
apy, including resistance to therapy-induced programed cell death,
or apoptosis, is necessary to combat therapy resistance.

The literature shows a myriad of chemotherapies that have
been trialed, mostly unsuccessfully, against RCC. Fluorouracil was
the preferred treatment for many years, in conjunction with other
chemotherapies like gemcitabine, and also with immunotherapies
like interferon (IFN) and/or the interleukins (IL) [42]. The main
immunotherapies that showed promise almost two decades ago
were IFN-α and γ and IL-2 and -12. They were eventually associ-
ated with low response rate and lack of effect on long-term patient
survival. Thus, offering a lot of initial hope, risk of patient death from RCC even when treated with the immunotherapies re-
ained high [43]. The use of immunotherapies, especially IFN, was
also limited at high concentrations by the toxicity to the normal,
non-cancerous kidney and the impact on patient quality of life. Ration therapy, as a treatment modality, is rarely used with
RCC compared with other solid tumors [44]. Functionally, radiation
targets cell proliferation, killing cells via direct DNA damage and
double stand DNA breakage, and also by the formation of free rad-
icals, especially reactive oxygen species [45]. Potentially, this form
of cancer therapy may have been able to circumvent the MDR
mechanisms of resistance in RCC. In fact, however, radiotherapy
had limited effectiveness against RCC, in part because it targets a
proliferative cell population, known to be low in most established
RCC. Chemotherapy such as fluorouracil and cisplatin [46,47], and
immunotherapy such as IFN [48] are sometimes used prior to radia-
tion therapy of RCC to sensitize the cancer population to radiotherapy.

Recent therapies for the treatment of metastatic RCC makes
excellent use of molecular-targeted chemical agents, like those
that target receptor tyrosine kinases (RTK) or the pro-angiogenic
activity of VEGF, common in many RCC [49]. Two ATP-competitive
inhibitors of the class III and class V RTK, sorafenib and sunitinib,
and the ligand-competitive inhibitor of VEGF, bevacizumab, were initially used to target angiogenesis in RCC. Inhibitors of the mammalian target of rapamycin (mTOR) gene, like temsirolimus and everolimus, target the control of RCC cell proliferation, survival, mobility and angiogenesis. Alone, or in combination with IFN-α, these drugs have significantly boosted progression-free survival in RCC, but complete remission remains elusive. Bortezomib is one of a new class of therapeutic agent targeting the 26S proteasome of the ubiquitin–proteasome degradation system [50]. The ubiquitin–proteasome pathway helps regulate cell cycle and development and growth of tumors like RCC. Bortezomib alters the degradation of regulatory cell cycle control proteins as well as interfering with gene expression, cell adhesion molecules, metastasis, and angiogenesis. In a trial of its use in metastatic RCC [50], only some patients responded, epitomizing the problem of the heterogenic nature of response to therapy in RCC patients.

Targeting apoptosis in resistance of RCC to treatment

Apoptosis was first described as a mode of cell death that is essential for tissue and organ development and homeostasis, with its dysregulation contributing to the pathogenesis of many diseases. The process aims to delete cells that are detrimental to an organism or tissue, such as pre-cancerous cells that have a mutation in their DNA [6,9]. Derepression of apoptosis may lead to cancer development and may also determine the response of cancers to different treatments. The investigation of mechanisms of resistance to these therapies, in particular the apoptotic mechanisms of resistance in RCC, remains an important area of research.

Most information on apoptosis in RCC relates to the two evolutionarily-conserved apoptotic pathways: the extrinsic pathway which utilizes cell surface death receptors; and the intrinsic pathway which involves the mitochondria and the B-cell lymphoma-2 (Bcl-2) gene family of pro- and anti-apoptotic proteins [51–54]. A family of cysteine proteases called the caspases (from “cysteine-aspartic acid proteases”) also determine levels of apoptosis [55]. In the extrinsic pathway, death ligands like TNF-α and TRAIL interact with their own death receptors [12] to stimulate recruitment of adaptor proteins. Brooks and colleagues investigated the ability of the drug bortezomib to sensitize RCC to induction of apoptosis by TRAIL. They reported that, in successful treatments, the drug caused early caspase-8 activity, setting off a caspase cascade to apoptosis. However, in the absence of increased caspase-8 activation, other bortezomib-induced changes were not sufficient to sensitize RCC to TRAIL-mediated apoptosis [56].

In the intrinsic pathway, there are many disparate published results with regard to the relevance of the Bcl-2 gene family and levels of apoptosis in the development and progression of RCC. Some researchers have found no correlation [57]. In contrast, in an immunohistochemical study of patient samples, RCC with increased expression of anti-apoptotic Bcl-2 and/or Bcl-XL had minimal levels of apoptosis, perhaps indicating a contribution to progression of RCC and resistance to treatments [58]. The caspases are involved in both the intrinsic and the extrinsic pathways. Wu and colleagues [59] found caspase-3 was cleaved, or activated, in fluorouracil-induced apoptosis in RCC. Davidson and colleagues also investigated the role of caspase-3 activation in resistance of RCC to apoptosis [60]. Cleaved caspase-3 was not detected by Western immunoblots in chemotherapy-treated RCC in culture. Caspase inhibition caused a reduction in, but not negation of, therapy-induced apoptosis. These results indicated that apoptotic pathways must occur in RCC but these pathways may not involve caspase-3 cleavage. Identification of caspase inactivation or redundancy specific to RCC may explain, in part, the resistance of RCC to cancer therapies and may be useful in targeting apoptotic pathways to overcome resistance to treatment of RCC. However, targeting these apoptotic pathways for treating RCC has, in general, been disappointing and investigation of alternative apoptotic pathways needs investigation.

TRAF as a novel molecular marker and treatment target for RCC

The molecular mechanisms of RCC development and progression are being explored to develop promising novel therapeutics. At present, no available compounds, used either singly or in combination, are capable of producing complete remission in metastatic RCC. The systemic toxicity of the therapies and resistance of RCC to treatment, and the higher cost of therapy associated with novel drugs, are all barriers to their use for the general population. In addition, genetic analysis of cancer cells indicates there is a much larger level of genetic alterations than suspected previously. Numerous subclones have been found within tumors and these are likely to have distinct adaptive potential to therapy. Recent research indicates that molecular mapping of individual patient tumors may become necessary, with treatments based on a molecular map, targeting multiple molecular pathways simultaneously to produce a better clinical outcome. Thus, there is a need for identification of effective molecular targets, especially for treatment of a cancer like RCC that shows great heterogeneity.

The TNF family proteins, specifically the TRAFs, were selected for this hypothesis paper because they have received limited attention in RCC to date, but they have demonstrated altered expression in development of other cancers [13,14], and are considered potential targets for treatment of other cancers [15]. TRAFs are primarily involved in the regulation of inflammation, antiviral responses and apoptosis [17]. They link the TNFR subfamily to signaling cascades. TRAF proteins are expressed in normal and diseased tissue in a regulated fashion, implicating a role in regulating physiological and pathological processes [19]. There are currently seven TRAFs (TRAF1–7) that are characterized in mammals and they share a relatively conserved secondary structure [19,61]. The TRAF domain found in TRAF1–6 consists of a bundle of eight β strands and one α-helical segment that form coiled–coil interactions. This domain mediates interactions with other signaling components, such as the transmembrane TNFR [19]. TRAF7 does not contain a TRAF domain, but interacts with the TNFR and so is included in this family [61]. The involvement of most of the TRAFs in inflammation [17] may be both good and bad for cancers. If the TRAFs act in a pro-apoptotic manner against tumor cells, then the demise of the cancer is not likely to stimulate inflammation and cause increased normal tissue damage through, for example, induction of reactive oxygen species and oxidative stress. If, however, they act to cause the demise of our natural immune defence against cancers, then loss of the immune surveillance will allow the cancer to grow. The TRAF-inflammation interaction has not been clearly defined for any cancers.

TRAF1 associates with TNFR2, forming homodimers and heterodimers [62]. Transgenic mice have been developed with targeted expression of a lymphoma-associated NF-κB2 mutant (p80HT) in lymphocytes. These lymphocytes had high survival rates and expressed high levels of TRAF1. TRAF1 knockdown abrogated the anti-apoptotic activity of p80HT and re-established B-cell homeostasis [63,64]. The significance of these interactions was not clear but the interaction partners were often anti-apoptotic in nature in those cancers. TRAF2, TRAF5 and TRAF6 also mediate activation of the key transcription factor NF-κB via various pathways, and also activate apoptotic pathways involving JNK and p38MAPK [65]. TRAF2 is currently the most studied of the TRAFs. It is a key adaptor protein transducing signals that emanate from many members of the TNFR superfamily. TRAF2 phosphorylation initiates molecular pathways that determine activation of NF-κB pathways.
Although the role of NFTosis, that is, TRAF1 might be considered pro-apoptotic [70]. The increased protein expression along with the therapy-increased apoptosis of TRAF1 in RCC development and treatment has been investigated; however, TRAF2 has received no attention in these cancers. Likewise others of the TRAFs may be relevant in RCC development and need further study.

We were interested in developing a clearer image of the role of the TRAFs in cancer to see how the expression of the gene coupled with disease process. Our specific interest was RCC. We have now developed some data that support a role for TRAFs in RCC development and therapy outcome. We had used the ACHN RCC cell line to analyze molecular pathways involved in apoptotic resistance of RCC to therapy [60,70]. Apoptosis-regulatory genes in an RNA microarray were compared in untreated and therapy-treated apoptotic RCC. Several genes, including TRAF1, TRAF3 and TRAF4, but not TRAF2, had significant up-regulation in therapy-treated RCC. Only TRAF1 was found subsequently to have correspondingly increased protein expression along with the therapy-increased apoptosis, that is, TRAF1 might be considered pro-apoptotic [70]. The functionality of TRAF1 in the RCC has now been tested using siRNA and TRAF1 and TRAF2, and not TRAF2, had significant up-regulation in therapy-treated RCC. Only TRAF1 was found subsequently to have correspondingly increased protein expression along with the therapy-increased apoptosis, that is, TRAF1 might be considered pro-apoptotic [70].

A parallel investigation was carried using immunohistochemistry for TRAF1 in 121 patient samples of RCC versus paired normal kidney. The samples were collected at nephrectomy for RCC but prior to any cancer therapy. Most were ccRCC (N = 95). The others were papillary RCC (N = 11), chromophobe RCC (N = 6), collecting duct RCC (N = 4), and unclassified RCC (N = 5). For statistical comparisons, the RCC cases were separated into ccRCC and "other RCC." We found that all samples (normal and RCC) expressed similar TRAF1 to varying intensities. Normal kidney consistently had strong expression in the proximal tubular epithelium, the segment of the nephron from which most RCC originate. Expression of TRAF1 was significantly reduced in all RCC, with ccRCC being lowest of all (p < 0.05 compared with "other RCC" and p < 0.01 compared with normal kidney) [Rajandram et al. manuscript submitted]. There was some variability in expression of TRAF1 in the RCC from different patients, but there were clear statistical differences between expression in normal kidney and RCC. The patient variability did not appear to be related to any inflammation that was recognized in the cancer samples. Whether or not low TRAF1 contributes to the growth of RCC and to their resistance to therapy through low apoptotic levels remains hypothetical. However, investigating TRAF1 expression in a particular patient's RCC and knowing that a certain therapy up-regulates TRAF1 in RCC with tolerable toxicity to normal kidney may lead towards successful patient-specific pro-apoptotic therapy.

Targeted TRAF1 protein therapy in RCC might improve success in treatment of this cancer. A goal of targeted therapy is to restore endogenous death pathways and thus drive cancer cells to destruction by apoptosis. One well-known example in the TNFR superfamily is the targeting of TRAIL receptor activation using recombinant human TRAIL. ligands or agonist antibodies against its death receptors DR4 and DR5, for tumor specific cell death [71,72]. Whilst there has been limited success with TRAIL receptor activation, even when used with other synergising anti-cancer agents like bortezomib [73], further in vivo testing should be carried out before this strategy is regarded as lacking worth [74]. If TRAF1 were used as a targeted therapy, the strategy would likely need to involve stabilized protein therapeutics. Without stabilization, injected proteins have a short in vivo half-life, physical and chemical instability, and low oral bioavailability, necessitating frequent injections of the protein. TRAF1 protein might be encapsulated in, and released slowly from, microspheres made of biodegradable polymers that are injected into the target site [75]. Recently, however, novel delivery molecular scaffolds have been identified for protein-based therapeutics. These are stable, biologically active, able to cross the cell membrane, and can be used to develop new, injectible, stabilized protein therapeutics [76]. Obviously, a therapeutic strategy such as targeted TRAF1 therapy may set up selective pressures for those tumor cells that can survive and proliferate in its presence by evolving mechanisms of resistance, including regulation of alternate anti-apoptotic pathways. For TRAF1 treatment strategies, much more validation of the worth of these developments would be necessary before commitment to the extensive research and substantial funding necessary for their development for human therapies.

Probably the greatest benefit of defining a role for TRAFs in RCC growth and development is their potential role in a biomarker panel for diagnosis or prognosis of RCC. There is currently an exponential increase in publications that discuss panels of biological markers for prognostic or predictive decisions on therapeutic procedures for various cancers. These markers should be stable molecules that can be measured objectively, preferably cheaply and easily, from patient serum, urine and tissue [77,78]. The main reason for the use of a panel of biological markers in any cancer is to provide a "story" of cancer development that may allow individualizing treatments or determining likelihood of survival. Prognostic markers aim objectively to indicate the patient’s clinical outcome independent of treatment. Predictive markers are associated with tumor sensitivity or resistance to therapy, and with that knowledge aim to predict the response of a patient to a specific therapy. For example, VEGF and HIF-1α correlate with prognosis in advanced stage RCC tumors. VEGF is the currently one of the most important independent prognostic factors for cancer-specific death, and in RCC tissue samples, the immunohistochemical profile of VEGF can be used successfully as an indicator of disease recurrence and survival of patients [79]. In another investigation where proteomic profiles of patients with metastatic RCC were analyzed, novel serological proteins associated with patient prognosis were identified [80]. Using surface-enhanced laser desorption ionization time-of-flight mass spectrometry, of the 10 proteins found to be associated with overall patient survival, apolipoprotein A2, serum amyloid alpha, and transthyretin were validated for overall survival from RCC metastases. By using the first two of these factors, a two-protein signature of overall patient survival was identified. With further validation, this two-protein signature has the potential to improve current risk stratification in RCC patients with metastases. Swanton and colleagues reported on a multi-disciplinary Personalized RNA-interference to Enhance the Delivery of Individualized Cytotoxic and Targeted therapeutics (PREDICT) study to develop predictive biomarkers of individual patient response to anti-cancer agents, especially those with anti-angiogenic activity like sunitinib and everolimus [81]. They analyzed tumor tissue from pre-operative RCC patients and used established and novel methods to integrate tumor-derived genomic data with personalized tumor-derived small hairpin RNA and high-throughput small interfering RNA screens. These methods aimed to identify molecular pathways important for survival and growth of RCC, and particular targets suitable for individualized therapeutic development. The work was very dependent on our understanding of molecular mechanisms driving resistance to the anti-angiogenesis agents, for which molecular interference of apoptosis may play a role, and also dependent on the current limitations of laboratory and clinical analyses. However, the investigation did show the worth of testing hypotheses about new therapies and predictive markers. We are
now testing our hypothesis that TRAF1 will also add value to current prognostic modeling and, possibly, decisions on appropriate therapies. We are using patient serum, urine and tissue samples from an increased patient cohort than had been used in our previous studies to develop these data.

Summary

RCC is the most lethal of the common urologic malignancies. Local RCC may be effectively excised surgically, or treated with cryoablation, with complete cure probable when detected early. However, metastasis is common and may have already established even with early diagnosis. Metastases are difficult to treat, and recurrences of RCC are common after metastases are thought to be removed. In addition, the ability of the kidney for functional compensation when part of it is destroyed means that early detection from loss of function is often not possible. One of the hallmarks of treatment-resistant tumors is a failure in initiation of apoptosis after treatment. Understanding the particulars of how cells fail in the apoptotic process is complex, and very tissue specific, and needs further study in RCC development and progression. The TRAFs have been shown to have prognostic and therapeutic potential in cancers other than RCC, and our preliminary data indicate they are worthwhile of further study in RCC. Prognostic and predictive markers, in principle, improve the chance of early diagnosis of cancers, importantly detecting cancers at their earliest and most treatable stages. However, to date, there is no adequate panel of specific molecular tumor markers for the differential diagnosis of RCC. Consequently, studying novel RCC apoptotic pathway genes, like the TRAFs, may help with the development of markers for RCC and also allow development of more targeted therapies against RCC.

Conflict of interest

None of the authors has a conflict of interest with any content in this manuscript.

References


