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Cancer, Carcinogens and Screening in the Kidney

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Abstract

This chapter examines a number of different aspects of renal cancer. Firstly, an introduction into the numerous forms of renal cancers is provided, focusing on renal cell carcinomas their causes and the different treatment options currently available. This chapter also takes a look at renal cancer from a toxicological point of view. Due to the crucial role of the kidneys in blood filtration, this allows them to become susceptible to the exposure and accumulation of potentially carcinogenic chemicals. For this reason, renal carcinogens are looked at in detail focusing on the varying mechanisms of genotoxic renal carcinogens such as aristolochic acid and potassium bromate, and their non-genotoxic renal carcinogens counterparts including ochratoxin A and chlorothalonil. This chapter also examines the different methods currently used to detect a compound's carcinogenic potential, including the *in vitro* Ames test and animal based carcinogenicity screening methods.

Keywords: cancer, renal cancer, renal carcinogens, carcinogen screening, genotoxic/non-genotoxic carcinogens

1. Renal cancer

In 2016 alone there was a reported 62,700 cases of cancer involving the kidney and the renal pelvis worldwide [1]. Renal cell carcinoma (RCC) is the most common form of kidney cancer and makes up approximately 90–95% of all kidney cancers [2]. The incidence of RCC has been found to be higher in developed countries and is also more common amongst men, the exact reasons for this are currently unknown [3]. Five year survival rates for stage I RCC is 80–90%, for stage II it is 80%, for stage III it is approximately 60% and stage IV survival is estimated at just 10%. RCCs can be categorised into a number of subgroups; clear cell RCC (ccRCC), papillary RCC and chromophobe RCC [4].

Clear cell RCC (ccRCC) aptly named due to its clear cytoplasm. This clear cytoplasm is caused by its high glycogen and lipid content in the cytosol. ccRCC is the most common form of RCC, making up 70% of all kidney cancer cases [5]. ccRCC are thought to arise from proximal tubular epithelial cells and have a worse prognosis than papillary or chromophobe carcinoma [6, 7]. Approximately 95% of ccRCC are sporadic with the remaining 5% having a familial link [7]. Von Hippel–Lindau (VHL) disease is a hereditary condition that is heavily implicated in the development of familial ccRCC [8]. Mutations in the VHL are implicated in virtually all cases of familial ccRCC and approximately 57% of sporadic ccRCC [9]. Mutations in VHL also lead to the activation of hypoxia-inducible factor-1 α (HIF1A) and hypoxia inducible factor-2 α (HIF2A). Activation of these HIF related pathways leads to the activation of genes involved in angiogenesis and vascular endothelial growth [6]. The second most commonly mutated gene in ccRCC is polybromo 1 (PBRM1). Mutations in this gene are found in 45% of all ccRCC, its exact role in the development of tumour growth is not well understood, however it is thought that PBRM1s role is in controlling cell proliferation [10]. Components of the PI3K/AKT pathway are also believed to be implicated in ccRCC. In a study of a ccRCC database of 20 PI3K/AKT pathway panel components were assessed, 27% of the components were found to have genetic alterations related to PI3K/AKT pathway [11].

2. Other renal carcinomas

Similar to ccRCC papillary renal carcinomas are thought to arise from the epithelial cells in the proximal tubule [6]. Two hereditary conditions are linked with the development of papillary renal carcinomas, these conditions involve mutations in the MET proto-oncogene and fumarate hydrate genes, respectively [4]. However, this form of renal carcinomas is not well understood.

Chromophobe RCC (chRCC) makes up about 5% of all renal cancers [7]. chRCC is usually found in stage I or II and the tumour usually presents itself as a highly lobulated large mass, with a median tumour size of 6 cm [12]. The exact genetic alterations in chRCC are not well understood, however loss of entire chromosome 2, 10, 13, 17 and 21 occurs in almost all cases of chRCC. It is the least aggressive of all RCCs and for this reason has a low malignancy potential with a 10 year survival rate estimated at 80–90% [13]. Tumour development can also occur in the collecting duct of the kidney. This is a rare form of renal cancer that usually presents itself at an advanced stage and is believed to arise from the epithelial cells in the collecting duct [14].

3. Treatment

Fifty percent of all renal cancers are diagnosed when an ultrasound is carried out for symptoms including abdominal pain, hypertension, weight loss and elevated CRP, due to this difficulty in diagnosing renal cancer about 25% of patients present with cancer that has metastasized [15]. Surgery is commonly used in the treatment of renal cancer, depending on the severity of the disease the nephrectomy can be partial or radical. Radical nephrectomy involves the removal of the entire kidney, the adrenal gland and possibly the regionally located lymph

nodes [16]. Although it is seen as an effective treatment of renal cancer, its use is associated with development of chronic kidney disease (CKD) and other cardiovascular related diseases [17]. Partial nephrectomy (also called nephron-sparing surgery) is also used, however this technique is underused due to the technical difficulties involved in removing only a part of the kidney where the tumour is localised. Partial nephrectomy has a 5 year survival rate of 87–90% [2]. Renal cell carcinomas are highly resistant to chemotherapeutic agents, for this reason the currently available drugs involve targeted therapies, including cytokines, mTOR inhibitors and targeting the vascular endothelial growth factor (VEGF) pathway [4]. A number of different cytokine related therapies are available such as interleukin 2 (IL-2) and interferon alpha (IFN-alpha), although these options have anti-cancer ability they are found not to be effective in cases where the tumour has metastasized [18]. The development of RCC is closely linked to a number of different components involved in angiogenesis, for this reason anti-angiogenic therapies have been developed targeting in particular the VEGF pathway, these include bevacizumab, sorafenib and sunitinib. These treatments have replaced interleukin related immunotherapies as a first line treatment following the partial or radical nephrectomy [19]. The mTOR pathway is involved in cell survival and its dysregulation has been shown to be heavily implicated in a number of forms of cancer [20]. Temsirolimus and everolimus, both mTOR inhibitors have been approved by the FDA for treatment of RCC, temsirolimus is considered the first line of treatment for metastatic RCC where the prognosis is poor, everolimus is used where the disease has progressed during treatment with VEGF targeted treatment [21].

4. Carcinogens

In the international agency for research on cancer (IARC) review of human carcinogens, they describe 'the term carcinogenic risk...is taken to mean that an agent is capable of causing cancer' [22]. Carcinogens are capable of inducing the process of carcinogenesis by variety of different mechanisms, these include by genotoxic (directly interacting with DNA) and non-genotoxic (indirectly lead to DNA instability) mechanisms. Carcinogenic factors have been grouped into these different categories; primary and secondary determining factor and favouring factors. Primary determining factors can be defined as a compound or chemical which is capable of inducing cancer by acting on a molecular level. Secondary determining factors are caused by hereditary factors where there is a genetic mutation with a familial link that ultimately results in cancer formation. Lastly, favouring factors are ones that may increase the possibility of tumorigenesis, these include diet, gender, age and possibly geographical location [23]. As well as carcinogens, there are also compounds called co-carcinogens that do not cause cancer by themselves but may increase the carcinogenic ability of other carcinogens [24].

5. Genotoxic vs. non-genotoxic carcinogens

Carcinogens can be classified by their mechanism of carcinogenicity. These categories include genotoxic and non-genotoxic carcinogens (**Figure 1**). In general, genotoxic carcinogens function

Figure 1. Mechanisms of genotoxicity and non-genotoxicity carcinogenicity. Carcinogens can be categorised as either genotoxic or non-genotoxic carcinogens. Genotoxic carcinogens induce the carcinogenesis process by directly interfering with DNA leading to genetic instability, if DNA replication occurs prior to the repair of the damage. Non-genotoxic carcinogens on the other hand indirectly interfere with DNA, this can occur by a number of mechanisms including ROS production.

by directly interfering with the patient's DNA, this causes the formation of covalent bonds and eventually leads to the development of DNA adducts, while non-genotoxic carcinogens do not directly interact with DNA but act in a carcinogenic fashion through other mechanisms. Genotoxic carcinogens are believed to induce DNA damage by the formation of a cross-linking bond between two helices, the removal of DNA bases and the cleavage of DNA strands, all contributing to the alteration of DNA [25]. In general, this DNA damage would be repaired, however if DNA replication occurs before the damage is fixed the mutation may result in cancer development. As all cancers have alterations in DNA expression, non-genotoxic carcinogens are capable of inducing this genetic instability by an indirect manipulation of the natural regulation of DNA expression. In general non-genotoxic carcinogens appear to be more specific than genotoxic carcinogens in their carcinogenic ability, where they are often only capable of inducing cancer in one species, in one organ and sometimes in one sex [26]. Although the mechanisms of non-genotoxic carcinogenicity are less well understood than their genotoxic counterparts, attempts have been made to classify the main mechanisms of non-genotoxic carcinogenicity, these have included receptor activation, CYP450 induction, stimulation of oxidative stress, chronic cell injury, immunosuppression and interference with intercellular communication [26, 27].

6. Genotoxic carcinogens

Examples of genotoxic renal carcinogens are potassium bromate, 2-nitrofluorene, benzo-A-pyrene, aristolochic acid and streptozotocin (**Figure 2**). Potassium bromate is a white crystal powder that is listed by the IARC as a group 2B carcinogen. Before its toxicity was established potassium bromate was widely utilised in the food industry, typically used to strengthen

Figure 2. Chemical structure of a number genotoxic renal carcinogens (All chemical structures are adapted from <https://www.ncbi.nlm.nih.gov/pccompound>).

dough. Potassium bromate is a highly toxic compound that can cause oxidative DNA damage by inducing the formation of 8-hydroxyguanine (oh8Gua) [28]. 2-nitrofluorene is found in the emissions from diesel fumes and kerosene heaters. This by-product of combustion is listed as a group 2B carcinogen. It has been shown to be capable of causing DNA adduct formation in rats, thus proving its genotoxicity [29]. Benzo-A-pyrene is another genotoxic carcinogen, listed as a group 1 human carcinogen. It causes a missense mutation in the tumour suppressor p53 [30]. Aristolochic acids are an extract from a plant commonly found in Asia. Plants containing aristolochic acids are classified as group 1 human carcinogens. Aristolochic acids are capable of producing DNA adducts found in tumour tissue of patients with urinary tract cancer in an area of Taiwan where the use of herbal medicine containing aristolochic acids is the most prevalent [31]. Lastly, streptozotocin is a 2B carcinogen with possible carcinogenic ability in humans and has been shown to cause tumour formation in rat kidneys and neoplastic transformation in human primary renal cells [32, 33].

7. Non-genotoxic carcinogens

Non-genotoxic renal carcinogens include ochratoxin A (OTA), monuron, bromodichloromethane and chlorothalonil (**Figure 3**). Ochratoxin A (OTA) is classified by the IARC as a group 2B carcinogen with possible human carcinogenic ability. OTA is a naturally occurring

Figure 3. Chemical structure of a number of non-genotoxic renal carcinogens (All chemical structures are adapted from <https://www.ncbi.nlm.nih.gov/pccompound>).

mycotoxin, OTA causes contamination of food and drink products [34, 35]. OTA exposure is associated with Balkan endemic nephropathy that causes tumour formation in the urinary tract and the kidney [36]. Much work has been carried out to establish whether OTA is a genotoxic or non-genotoxic carcinogen. The formation of DNA adducts following OTA treatment would point to a possible genotoxic mechanism [37]. However, a review by Mally et al. refuted these findings where they claimed that OTA induced DNA adduct formation was not an important mechanism in OTAs overall carcinogenicity [38]. A study by Mantle et al. discussed that the reclassification of a carcinogen from a non-genotoxic to genotoxic has far reaching ramifications in terms of legislation, particularly with a contaminant found in the food and drinks industry, as a non-genotoxic carcinogen is said to have a 10-fold reduced risk to humans compared to genotoxic carcinogens [39]. This being said OTA's ability to induce the formation of reactive oxygen species is the strongest piece of evidence to suggest its non-genotoxicity [40].

Monuron is a phenylurea herbicide and a known renal carcinogen in rats as it has been shown to cause adenomas of the kidney and carcinomas of the liver [41]. In a study by Block et al. they showed that monuron induced an upregulation of genes involved in cell cycle and cell proliferation in the renal cortex [42]. Due to its unclear mechanism of carcinogenicity a number of studies have classified it as a non-genotoxic renal carcinogen [43]. Bromodichloromethane is a trihalomethane and according to the IARC is a proven carcinogen in rats and a suspected human carcinogen (Group 2B). It is also thought to cause some chromosomal aberrations

[44]. Chlorothalonil is a fungicide used in fungal control in a number of different crops. It is classified as a group 2B carcinogen to humans. A report by the *Environmental Protection Agency* in the United States declared that although an exact mechanism of carcinogenicity for chlorothalonil was unestablished it was agreed that the probable mechanism of carcinogenicity was through a non-genotoxic route as it behaved very similarly to other well established non-genotoxic carcinogens [45].

8. Bioactivation of carcinogens

In general cytochrome p450 monooxygenases play the role of detoxifying certain chemicals in the body, the opposite can also occur where these enzymes can bioactivate a particular compound where oxidation causes the conversion into a toxic by-product [46]. 2-acetylaminofluorene, a carcinogen capable of inducing tumour formation in the liver and kidney, is converted by a CYP450 mediated N-hydroxylation to produce a hydroxylamine which undergoes further transformation to become the deadly activated carcinogenic form [47]. Another example of CYP450 induced bioactivation, is the conversion of genotoxic benzo-A-pyrene to the carcinogenic benzo-a-pyrene-diol-epoxides by the enzymatic action of CYP1A1 and CYP1B1 [48]. Other families of enzymes can also carry out a similar function in converting carcinogens to their active form, including sulfotransferase. Sulfotransferase family cytosolic 1B member 1 (SULT1B1) converts the genotoxic carcinogen aristolochic acid to its active form by the action of sulfotransferase enzymes specifically SULT1B1 [49].

9. *In vitro* carcinogenicity screening

The Ames test is a bacterial mutagenicity assay, which is designed to be able to detect a chemical's ability to induce DNA damage that leads to gene mutations. The Ames test was first developed by the work of Bruce Ames, where he attempted to detect chemical mutagens with the use of bacteria [50]. This now well established assay works with the use of different strains of *Salmonella* that have mutations in genes involved in histidine synthesis, this causes the bacteria to be unable to grow [51]. The principle of the Ames test is based on whether a chemical can induce mutations that can cause the bacteria to again produce histidine which would then allow bacterial growth. To allow for the detection of various mutagens that function by different mechanisms different strains of *salmonella* are used that have varying mutations in the histidine operon [51]. Over the years the Ames test has undergone a number of modifications from the original method [52]. The most significant improvement was the incorporation of rat liver microsomes, which allowed for the screening of the original compound undergoing testing and any potentially carcinogenic metabolites that could be produced [53]. In more recent years human liver S9 microsomal fractions have been utilised to try and improve the ability to detect carcinogens in humans. The main limitation of the Ames test still remains, which is *Salmonella typhimurium* being a prokaryote, however it is still useful as an initial carcinogenic screen.

Other *in vitro* screens for identifying carcinogens are collectively called cell transformation assays (CTAs). CTAs were first developed in the 1960s, in a study by Berwald et al. they showed that a carcinogenic hydrocarbon (Benzo-A-pyrene) was able to cause a transformation from normal cells to tumour cells [54]. The three main assays used nowadays are the BALB/c 3 T3 assay (mouse embryo cells), the Syrian hamster embryo cells (SHE) assay and C3H10T1/2 assay (pluripotent stem cells) [55]. The Organisation for Economic Co-operation and Development (OECD) carried out a very comprehensive review of the BALB/c 3T3, SHE and C3H10T1/2 assays. Each of the assays were assessed based on a number of criteria including sensitivity, specificity and predictivity. The C3H10T1/2 assay had the highest predictivity percentage of human carcinogens at 95%, this was followed by SHE and BALB/c 3T3 assays that had predictivity of 88 and 77% respectively [56]. Being able to predict the carcinogenicity of non-genotoxic carcinogens has been a major problem in carcinogenic screening, this has been somewhat overcome with the use of CTAs as the transformation of cells occurs with both genotoxic and non-genotoxic carcinogens [57]. As well as being used as screens for carcinogens CTAs are also used in investigating tumour initiation, evaluating classes of chemicals and establishing mechanism of action of compounds [55].

In 2006, the EU introduced the registration, evaluation, authorisation and restriction of chemicals (REACH) initiative. The implementation of these policies highlights the needs for an improved protection of human and environmental health and safety by the identification of potentially dangerous chemicals and their exact mechanisms of hazardness. These regulations have been phased in gradually over an 11 year period since their inception [58]. This has led to much stricter rules and regulation being put in place for the chemical industry and the need for improved carcinogenicity screening methods. Although very useful in predicting carcinogenic potential, the limitations of *in vitro* testing methods must also be recognised. In a study by Walmsley et al. they highlight the challenges involved in *in vitro* screens where non-carcinogens can sometimes be determined as carcinogens and the difficulties this can cause [59].

10. Animal models based carcinogenicity screening

A 2 year rodent bioassay is widely used in carcinogenicity risk assessment. The goal of this screening method is to be able to predict potential carcinogenicity, while also characterising any potential tumour development. Before the 2-year study commences either or both 14 or 90 day pre-chronic study must be completed to establish maximum tolerated doses [60]. The *National Toxicology Programme* set out a protocol in 1976 that is still used today. This protocol involves the use of 50 animals per sex per group [61]. The testing system is categorised into acute, sub-chronic and chronic exposure and in some cases *in utero* exposure. The study concludes with a histological assessment to identify any potential carcinogenic related changes. Attempts have been made to improve on the traditional 2-year rodent method, one such suggestion by Cohen et al. proposed that the 2 year study should be replaced by an enhanced 13 week screen [62]. This study suggests a shorter more robust study is sufficient to predict carcinogenicity, thus reducing the number of animals used, as well as time and costs.

The proposed screening process involves a tiered approach where the first step is to establish genotoxicity, estrogenic/immunosuppressive activity, this would then be followed by a multi dose response screen of the compounds effects on cell proliferation and overall toxicity. However, this shortened screen could fail to predict the carcinogenicity of a chemical where the effects take longer than 13-weeks to emerge. The main arguments against the use of the well-established 2-year rodent bioassay will always be whether this particular animal is suitable for predicting disease in humans and the issue of the unnecessary use of animal models. These limitations have been further highlighted with the EU's commitment to 'reduce, refine and replace animal models' [63].

Due to the kidneys function as a blood filtration system, it allows for the exposure and accumulation of potentially carcinogenic substances, leaving the kidneys to be susceptible to the development of various forms of renal carcinomas. For this reason there is an ever increasing need to improve screening methods that are capable of detecting carcinogens particularly those known to induce the development of kidney cancers.

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