Cisplatin resistance in urothelial carcinoma

Valorisation

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'Knowledge valorisation refers to the process of creating value from knowledge, by making knowledge suitable and/or available for social (and/or economic) use and by making knowledge suitable for translation into competitive products, services, processes and new commercial activities.'

- adapted definition based on the National Valorisation Committee 2011:8

The socio-economic burden of cisplatin-resistant urothelial carcinoma

Bladder cancer (BC) is the most expensive cancer among the elderly and carries high lifetime treatment costs for each patient diagnosed with this disease [1]. Of all BC cases 90 % occur in the urothelium [2], at which 1 in 25 Western men and 1 in 80 women will be diagnosed with urothelial carcinoma (UC) sometime in life. Both sexes have the highest risk in developing the disease within 10 years at the age of 75. In contrast, in many developing countries life expectancy is much lower, which is one of the reasons why overall non-age-specific UC incidence is lower in these countries [3].

It is expected that the burden of UC will increase in less developed areas of the world. Worldwide differences can be attributed to global changes in exposure to risk factors for UC as well as growth and aging of the world population. Currently, more than 112 million cases in 78 countries are suffering from elevated chronic urinary infection caused by *Schistosoma haematobium*. In these regions, such as Egypt, squamous cell carcinoma was for many years the dominant histopathological type of UC [4]. Otherwise, in Western countries, occupational carcinogens and cigarette smoking are the most important risk factors for UC [3]. In particular the latter accounts for approximately half of all cases, since tobacco smoke contains aromatic amines that are known to cause UC [5]. Nevertheless, BC incidence and mortality show a decrease in Western communities over the last decades, which can be attributed to a shift in smoking behaviour. Smoking prevalence in Western Europe started to decline from the 1950s for men and from the mid 1970s for women. Consequently, the drop of the UC incidence in men and rise in women can be attributed to the change in smoking habit between sexes over a period of 30 years [5]. Since tobacco advertising has been reduced by law in most Western countries, the tobacco industry is now focusing on the developing world [3, 5]. Many low- and middle-income countries are still in the early stages of the tobacco epidemic. Consequently, an increase in UC incidence in these regions is expected in the next decades. Therefore, prevention of smoking should be of primary concern in developing countries [3].

A recent study calculated 3591 € as the average cost of illness per year per patient. Therefrom non-medical costs, which consider lost productivity from time spent in and recovering from treatment, account for about 29 % to 44 % depending on the country [6-8]. UC awareness can contribute to early diagnosis, which can reduce the cost of the disease [9].

For curative intent, patients who already have or progress to muscle-invasive BC (MIBC) are treated by radiotherapy, chemoradiotherapy, radical cystectomy, or cisplatin-based (cisplatin/ gemcitabine or methotrexate/ vinblastine/ doxorubicin/ cisplatin) neoadjuvant chemotherapy followed by radical cystectomy. Adjuvant chemotherapy is used in some patients [10]. Since neoadjuvant cisplatin-based combination chemotherapy improves overall survival in MIBC and is an established standard [11, 12], the development of resistance mechanisms remains a major obstacle in the clinic.

Public availability of research results

In this dissertation we explored the multifacial character of cisplatin resistance in UC. The data presented in chapters 2 to 6 have been accepted and published in peer-reviewed, PubMed-indexed, and influential (5-year impact factor > 3.5) journals. Additionally, chapters 2, 3, 5, and 6 have been communicated and presented to academic audiences in various seminars and (inter-) national conferences, as displayed in chapter 8. The data displayed in the publications can be used as a starting point and reference for future research, which will minimalise the chances of repetition. This is specifically true for 'negative data', as shown in chapter 5. There, we could not confirm CD90 as a potential stem cell marker in UC, even though it was previously stated by other groups [13]. Moreover, since chapters 2, 3, 4, and 5 were published with open access, knowledge is available not only to experts in the field, but also to everyone who is interested. Independently from the publishing policy, we included additional information and raw data to most of our manuscripts in supplementary online files. Only about 9 % of papers published in high-impact journals did deposit full primary raw data online. According to the authors, a statement of willingness to share raw data by the primary investigator did not always translate into true availability of data when requested by independent scientists. Data withholding is not uncommon in the research community and may be influenced by industry, perceptions of proprietary information, lack of resources, and personal investigator stances towards data sharing [14]. Therefore, these promising findings and discoveries may not be reproducible, which should be the foundation of good laboratory practice. Consequently, approximately 85 % of biomedical research is wasted at-large [15]. In this regard, biomedical research is the most prolific scientific field. It is practically impossible even for the expert to maintain direct knowledge of the work done by so many other scientists, even when it comes to his/her core discipline of

interest. The high pressure to publish is initiated by limited funding and resources. Investigators who are assured of more consistent, stable laboratory funding are less likely to succumb to the pressures of fraud and the earlier mentioned not sharing of research data [16].

In this context it is evident that open access articles are cited earlier and, on average, even more often than non-open access articles. Therefore, open access accelerates the scientific advancement and knowledge translation of research into practice [17]. Another possibility to make research more available and eventually reproducible, is the use of pre-publication release [18], such as bioRxiv [19] and arXiv [20].

Translating research knowledge to the clinic: from bench to bedside

Albeit cisplatin is known for four decades and hundreds of studies have been investigating resistance mechanisms in cancer, this is the first study to analyse several mechanisms in at least four pairs of cisplatin-resistant UC cell lines. This dissertation explored the unique networking between pathways and the broad picture of cisplatin resistance, which cannot be targeted by one factor only. Most interesting was the cell line dependency, which reflected the individual cisplatin response observed in patients in the clinic.

We identified and also excluded factors relevant in cisplatin-resistant UC cell lines. Morphological changes observed in cisplatin-resistant UC cell lines could be attributed to epithelial-to-mesenchymal transition and increased non-canonical WNT activity. Therefore, phenotypic plasticity rather than a repopulation of the potential cancer stem cell marker CD90 and CK14 led to cisplatin resistance in UC cell lines (chapter 5). Moreover, the decreased amount of Pt-DNA adducts (chapter 2) caused by increased detoxification via NRF2 (chapter 3) as well as decreased cell death by increased anti-apoptotic factors, such as Survivin (chapter 2), and increased autophagy (chapter 4) were the main mechanisms identified in cisplatin-resistant UC cell lines. These findings imply a chemotherapeutic approach that is not only cisplatin-based, but should be combined and include NRF2 and Survivin inhibitors, such as brusatol and YM155, respectively. However, the results obtained from the chicken chorioallantoic membrane model indicated drug-dependent differences in physiological and pharmacokinetic uptake (chapter 6), which should be taken into consideration in future *in vivo* and patient studies.

Involved risks using a 2D cell culture system: a critical view on in vitro data

The findings displayed in this dissertation were mostly performed in long-term cisplatin treated UC cell lines, which had to be passaged for several months under constant cisplatin stress. This procedure is error-prone due to the long-term handling of several cell lines, under similar conditions for a long period of time. Cell lines are frequently misidentified or contaminated by other cells or microorganisms. Since the 1960s, more than 400 widely used cell lines worldwide have been shown to have been misidentified. Moreover, long-term passaged cell lines can undergo chromosomal duplications or rearrangements, mutations, and epigenetic changes that alter their phenotype, which is somehow inevitable, but potentially problematic *in vitro*. Using short tandem repeat (STR) analysis to identify DNA sequences unique to a cell line is now widely available [21]. Even though our cell lines were verified, we cannot exclude cell line specific and cisplatin-independent changes due to long-term culturing.
Using a 2D cell culture model for most of the presented experiments is a major limitation of this study, as it is a confounding risk factor [22]. To avoid confounding as well as selection bias [22, 23], we increased not only technical, but also biological duplicates in several cell lines. Nevertheless, handling high-dose cisplatin-resistant UC cell lines that are under constant cytotoxic stress rather represents an extreme situation for the development of resistance mechanisms.

This study supports the future approach to investigate more deeply into cisplatin analogues with increased and longer affinity with DNA, such as 9-aminoacridine Pt-complexes [24] and platinum-nitroxyl complexes [25], to elevate and specify cytotoxicity.

In conclusion, once validated in murine xenograft models, the data presented in this dissertation have a potential to optimise cisplatin treatment in terms of its dosage length, intensity, time- and context-dependency. This could result in faster recovery in patients and measureable saving of the social and economic cost of the disease.

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