Targeting CAIX with small molecules

Valorization addendum

This thesis describes different dual target approaches to target hypoxic tumor-associated carbonic anhydrase IX (CAIX). Firstly, the design, synthesis and biological evaluation of nitroimidazoles combined with carbonic anhydrase IX inhibitors (CAIXi), which showed the potential of increasing the efficacy of conventional treatment modalities such as chemo- and radiotherapy, has been described. Secondly, the development, synthesis and evaluation of anti-cancer drugs in combination with CAIXi derivatives, targeting the hypoxic tumor microenvironment to decrease normal tissue toxicity, have been demonstrated. Lastly, the design, synthesis and evaluation of biological efficacy of bio-reducible drugs, which undergo a reduction under hypoxic conditions, incorporating a CAIXi moiety have been described. However, developing new anti-cancer drugs remains challenging, since cancer is an extremely heterogeneous disease consisting of distorted versions of a person's own cells. Moreover increasing cancer incidence has a severe socio-economic impact and the development of new anti-cancer drugs remains essential. This valorization addendum discusses CAIX targeting using different approaches, its value for the gain for general society and future of cancer treatment.

Clinical Relevance

Since the treatment of cancer patients is progressing into a personalized treatment, the knowledge about the hypoxic tumor microenvironment and heterogeneity plays an important role. Conventional therapies such as chemo- and radiotherapy are less effective towards hypoxic tumors and therefore patient prognosis is worse. Many biomarkers have been already identified to stratify patients for a personalized treatment. CAIX is a transmembrane enzyme, its overexpression is highly tumor-specific especially under hypoxic conditions and generally associated with worse prognosis for cancer patients. Therefore, CAIX might be considered as a valuable biomarker for stratification of cancer patients, although the specific treatment based on this type of stratification remains to be identified. Since CAIX expression under hypoxic conditions is highly cell line dependent, targeted CAIX imaging may not be a universal hypoxia imaging strategy, and therefore, would be of limited use for clinical practice. CAIX catalyzes the reversible hydration of carbon dioxide to bicarbonate and a proton, a chemical reaction implicated in several carcinogenic processes such as invasion and migration. The clinical benefits of using CAIX inhibitors in patients however remain to be investigated. The dual target compounds to target CAIX with different approaches described in this thesis (Chapter 3, 4, 5, 6) will unlikely be implemented in clinical practice due to limited efficacy. So far, there is only one CAIX inhibitor (i.e. SLC-0111) in clinical trials. The phase I clinical trial (NCT02215850) has been successfully finished end 2016. Currently the compound is scheduled to enter phase II trials this year.

Gain for Society

The Transmembrane location and the tumor-specific overexpression of CAIX can facilitate multiple targeting strategies. Firstly, since hypoxic tumors show resistance to conventional therapies such as chemo- and radiotherapy, a dual targeting approach

with radiosentizers targeting CAIX can increase the efficacy of standard treatment modalities. Secondly, treatment costs will decrease when healthy tissue side effects caused by anti-cancer therapy can be reduced, e.g. specific delivery of cytotoxic drugs to tumor site via targeting CAIX. Lastly, hypoxia activated prodrugs or bio-reducible drugs, which are activated and become cytotoxic under hypoxia, can also decrease side effects by killing specifically the hypoxic tumor cells. In general, any of these advancement in treatment of cancer has a potential gain for society.

Improvement in Health Care

CAIX expression in normal human tissues is rare, except in GI tract and this expression either decreased or lost during carcinogenesis. This atypical expression helps as biomarker for theragnostic purposes, inhibiting CAIX alone with inhibitors may not be enough to improve the health care. During the last decade, a large variety of CAIX inhibitors, several reported in this thesis, have been developed, but definitive conclusions regarding the efficacy of a single CAIX inhibitor treatment can only be drawn after completing clinical trials. Dual drug targeting approaches to increase the efficacy of conventional treatment modalities, sparing normal tissues by specific drug delivery to the tumor site and bio-reducible drugs that become cytotoxic only under hypoxic conditions are expected to improve health care as patients will experience less toxic side effects but required additional interventions (Chapter 3, 4, 5, 6). These approaches can potentially increase the therapeutic window of anti-cancer therapy and thereby result in an improvement in health care.

In addition, CAIX expression is tumor-specific and has prognostic value in a wide variety of cancers types. The expression levels of CAIX could therefore serves as excellent biomarker for patient selection, although the relevant therapies remain to be identified. CAIX might also be a diagnostic tool for early detection of malignant lesions, e.g. implementing CAIX imaging can lead to a great improvement in health care, since early detection of cancer can increase the patient's chance of survival.

Novelty of the Concept

Targeting CAIX with different inhibitors is not novel, but recently different approaches of dual drug targeting, as discussed in this thesis, gained a lot of interest by researchers. CAIX is a potential therapeutic target due to its tumor-specific expression and its involvement in maintaining a pH balance between the acidic extracellular and the alkaline intracellular environment of tumor cell. The acidic extracellular tumor microenvironment promotes several carcinogenic processes such as migration and invasion. By reducing this extracellular acidification via inhibiting CAIX activity using nitroimidazoles incorporated with CAIXi, the efficacy of conventional treatment modalities might increase (Chapter 3). The interaction between CAIXi and conventional treatment modalities needs to be understood very well before entering into clinical trials.

Although cytotoxic drugs targeting CAIX have been described previously, these approaches were studied predominantly in renal cell carcinoma where CAIX expression is upregulated due to a mutation in the VHL protein preventing HIF-1α degradation. This thesis described a novel method of delivering anti-cancer drugs

towards CAIX expressing cells via dual target compounds (Chapter 4), which conjugated with CAIXi. However, these dual target compounds showed little preference for CAIX expressing cells, which minimized their practical applicability. Further exploration on pharmacokinetic studies on these dual target drugs may help to design more potent approaches to deliver cytotoxic drugs towards CAIX expressing cells. This thesis describes a novel approach of bio-reducible cytotoxic warheads conjugated with CAIXi. Most of the bio-reducible drugs described in Chapter 5 did not show strong binding affinity towards physiologically relevant CA isoforms, this might be explained by the influence of the linker and substitution on aromatic ring. Chapter 6 described 2-, 5-nitroimidazole and nitrogen mustard sulfonamides based derivatives. Interestingly, a cell dependent cytotoxicity has been observed for the 2-nitroimidazole-based compounds, an effect explained by the higher reduction potential of 2-nitroimidazoles compared to 5-nitroimidazoles. 2-nitroimidazole bio-reducible drugs may serve as less toxic drugs towards normal tissues surrounding the tumor and further combination with radiation might increase therapeutic efficacy. Structure Activity Relationship studies on bio-reducible drugs conjugated with CAIXi might help to develop new anti-cancer drugs.

Road to the Market

CAIX is an interesting anti-cancer therapeutic target and the tumoral expression of CAIX may be promising for the future market. The research described in this thesis does have potential clinical relevance that could eventually lead to an improved health care. However, additional i*n vivo* preclinical and clinical experiments remain essential. The prognostic value, tumor-specific expression and surface location of CAIX facilitate it to be a potential biomarker. For this purpose, investigations on the outcome and progression of CAIX-positive hypoxic cancer versus CAIX-negative hypoxic cancer should be conducted. Immunohistochemical evaluation of CAIX expression requires a specific antibody to detect CAIX, currently the M75 antibody available in the market. However, the development of a fast and easy to use kit to evaluate CAIX expression might therefore more promising, which is currently unavailable. Many CAIX directed antibody-drug conjugated candidates were reported, but there is not yet an approved therapeutic drug targeting this antigen.

The CAIX inhibitors described in Chapter 3 however will not be pursued further as they were either ineffective as single agents, or unable to increase the efficacy of conventional treatment modalities. However, the single agent CAIX inhibitor i.e. SLC-0111 entered into phase II clinical trials and future clinical trials should also investigate the effect of SLC-0111 on the efficacy of conventional treatment modalities.

Monitoring of CAIX expression levels can be used to stratify patients for a specific therapy that has been proven to be effective for e.g. specific cytotoxic drug delivery to tumor site by targeting CAIX. Another approach is the use of bio-reductive drugs conjugated with CAIXi that might improve the drug uptake by tumors expressing CAIX with minimal side effects. In future, this approach might help the development of new anti-cancer drugs targeting CAIX with a potentially clinical relevance.

Overall this thesis described several dual target approaches to target CAIX, but the most promising approach to utilize CAIX as target are the targeted drug delivery of cytotoxic compounds and bio-reductive cytotoxic drugs conjugated with CAIXi. In addition, the identification of alternative approaches to target CAIX remain essential, but requires further preclinical and clinical research in order to assess CAIX as a therapeutic target and its influence on healthcare and gain for society.