Exploring the benefits of inhibiting HIF and Notch to overcome resistance to cancer therapy

Valorization addendum

Tumor biomarker selection and targeting is crucial when treating cancer patients with the ultimate goal of overcoming resistance to conventional therapy. Over the last decades, a whole new research on biomarker discovery and validation is being tested in preclinical and clinical studies, demonstrating that selective biomarker targeting is a highly active field of research. Targeting resistant cancer cells remains the main barrier we face in improving outcome. The remarkable ability of cancer cells to adapt to the new environment conditions is the most frustrating characteristic of therapy resistance. However, the introduction of new targeting strategies such as the CRISPR/CAS9 technology or the discovery of novel oncogenic genes helps developing new treatment options for patients. The main aim of this thesis was to evaluate several tumorigenic aspects of different oncogenic proteins while also combining its inhibition with the effect of radiation.

Clinical relevance

In this thesis, the scientific relevance of modulating the HIF-1/2α proteins and NOTCH in different cellular models and in the context of cancer treatment has been demonstrated. Scientific knowledge in cancer research allows us to better characterize the basic molecular mechanisms behind a tumorigenic phenotype but also to develop new technologies or treatment strategies that eventually benefit society. Nowadays, cancer disease is one of the foremost causes of death worldwide. The most common causes of cancer death worldwide include lung, liver, stomach and bowel cancer, accounting for almost half of all cancer deaths. Plenty of treatments are currently available to treat cancer, some of which are optimal in initially reducing tumor growth and even lead to cures, however, this positive outcome is not always long-lasting and some tumors may recur. There is a population of resistant cancer stem cells that we have aimed to tackle in this thesis by trying to inhibit the proteins that those cells express and thus improve the effect of radiation. Many biomarkers have been already identified to strategy patients for personalized treatment. Since many cancer patients receive radiation treatment, approaches that influence radiation therapy have a great impact on cancer lives.

HIFα proteins are transcription factors of which the stabilization and activity are often tumor-specific especially under hypoxic conditions and generally associated with worse outcome for cancer patients. Therefore, HIFα might be considered as a valuable biomarker for stratification of cancer patients, although the specific treatment based on this type of stratification needs further investigation. Evidence on the prognostic value of HIF-2α protein

in cancer patients can be seen in this thesis in a meta-analysis collecting survival data from previously published studies. HIFα has become a potential target for developing novel cancer therapeutics since early 1990s. These inhibitors target the expression and/or function of HIF-1α, HIF-2α, or both, through direct and indirect mechanisms. A number of HIFα inhibitors have been developed and although some of them are under investigation, monotherapy treatments with HIFα inhibitors have not been successful in clinical trials and many have been halted. However, these inhibitors might be beneficial in order to sensitize resistant cells to conventional treatment modalities such as radiotherapy. Indeed, in this thesis we have shown that double- HIF1/2α inhibition greatly improves radiation sensitivity. Nevertheless, we also showed that individually inhibiting HIF-1α in hypoxic cells might confer an opposite phenotype due to a compensation mechanism mainly triggered by HIF-2α overexpression. Potentially, this is applicable to other treatment modalities like chemotherapy or immunotherapy and thereby contribute to a more optimal treatment of patients.

NOTCH has been found to influence tumor sensitivity to therapy and normal tissue regenerative capacity. Both characteristics are essential to improve treatment tolerance in cancer patients by increasing the effectiveness of the therapy while sparing normal tissue toxicity. The using of NOTCH inhibitors seems to be a promising strategy to eliminate the resistant cancer stem cells of the tumor and has been tested in many clinical trials. Patients receiving NOTCH inhibition treatment however, show severe adverse effects which ultimately lead to the abrogation of clinical studies. In our thesis we described a stem cell proliferative advantage in primary bronchial epithelial cells upon Notch inhibition. Since radiotherapy damages the normal tissue, increasing the proliferative capacity of stem cells might benefit patients which undergo radiotherapy and thus broad the dose-effect in cancer cells. Of course, the effect on the tumor and its microenvironment still need to be carefully elucidated in patients when combined with radiotherapy. The toxic effects driven by NOTCH inhibition might also be reduced by targeting different components and combinations of the γ-secretase complex. Since NOTCH regulates various pathways necessary for proper tissue regeneration and homeostasis, assessing a more specific NOTCH treatment strategy is essential. This thesis demonstrated that it is utmost important to modulate the activity of NOTCH by targeting specific components of the γ-secretase complex.

Gain for society

Despite the novel advances on cancer therapeutics, prevalence of cancer and its mortality rate is still affecting human health while also influencing economic stability in the world. The long and sometimes frustrating process of drug development for treating cancer accounts for most of the costs of cancer in society. Preclinical data aims to identify a compound, describe its mechanism of action and eventually transfer it to the clinic for patient testing. Before entering the market most of the drugs are discarded due to elevated toxicity or lack of effects. This inefficient process of drug discovery for patient usage not only costs millions of dollars every year but also the people we love.

The observations made in this thesis could potentially benefit cancer patients and the society in general. First, a new way of stratifying patients based on HIF-2α expression gives more insight into the prognosis of cancer patients, thus better predicting the survival rate of patients. Patients with lower HIF2 expression maybe selected for less aggressive treatment because they have a better prognostic outcome. This thesis also investigates the application of a novel way of biomarker targeting by using the CRISPR/CAS9 in human cells, a unique and elegant genetic-engineering tool that efficiently targets and removes specific genes from the genome. More studies are needed to better characterize the effects of using CRISPR/CAS9 in human cells in terms of a live organism and ultimately its application in cancer gene modification in patients. Differences in HIF1/2α-dual targeting offers an opportunity to design more efficient HIFα-blockers and increase the radiation response in cancer patients. Also, our study found a compensation mechanism by HIF-2α that should be considered when treating patients with HIF-1α inhibitors. Moreover, previous investigations from our research group found a beneficial effect on targeting NOTCH in combination with conventional therapies in cancer, which in this thesis we also show it would help patients prevent toxic-effects in the normal tissue. To end, patients presenting oncogenic NOTCH might benefit from modulatory-NOTCH inhibition by using Psen2:Aph1B γ-secretase inhibitors while sparing total NOTCH inhibition-related effects. Together, these findings may result into more effective therapeutic ratio when using NOTCH inhibitors in cancer.

Road to the market

Although the discoveries of our work represent a potential clinical relevance with a gain for society and could eventually lead to an improved outcome for treatment of cancer patients, it's translation from bench to bedside for daily clinical practice needs further investigation. Our data indicates that HIF1/2α inhibitors are unlikely to radiosensitize tumors alone, but that

they can significantly increase their effectiveness when combined. Consequently, several clinical trials using dual-HIF1/2α inhibitors should be of interest to test how well these inhibitors are tolerated also when combined with chemotherapy and radiotherapy, and whether any added benefit is observed. For instance, clinical trials have reported that BAY 87-2243 was found to suppress the accumulation of both HIF1/2α in non-small cell lung cancer cells; however, a phase I clinical trial (NCT01297530) using such compound was terminated due to safety reasons. Other HIF inhibitors have been selected to treat solid tumors. Unfortunately, up to now, no drugs directly inhibiting HIF1/2α have been approved for treating cancer due to safety or limited therapeutic efficacy. Lack of patient selection might also contribute to clinical trial failure. When evaluating the efficacy of HIF inhibitors in clinical trials, patients with advanced solid tumors are recruited, regardless of HIF expression in tumor cells. In this case, HIF inhibitors might not show therapeutic effects for accounting with those with low HIF levels. Therefore, clinical trials should also be stratified for patients with different protein levels in order to improve the concept of personalized medicine in cancer treatment and reach a better therapeutic effect for a specific subpopulation of patients with elevated HIF levels. Also, advances in CRISPR/CAS9 in applied medicine might offer a new way of selectively and accurately targeting cancer cells in patients. In total, the process may take many years before this technology can be used in daily clinical practice.