Mapping of EGFR treatment effects and uncovering DNA repair mechanisms using quantitative proteomics

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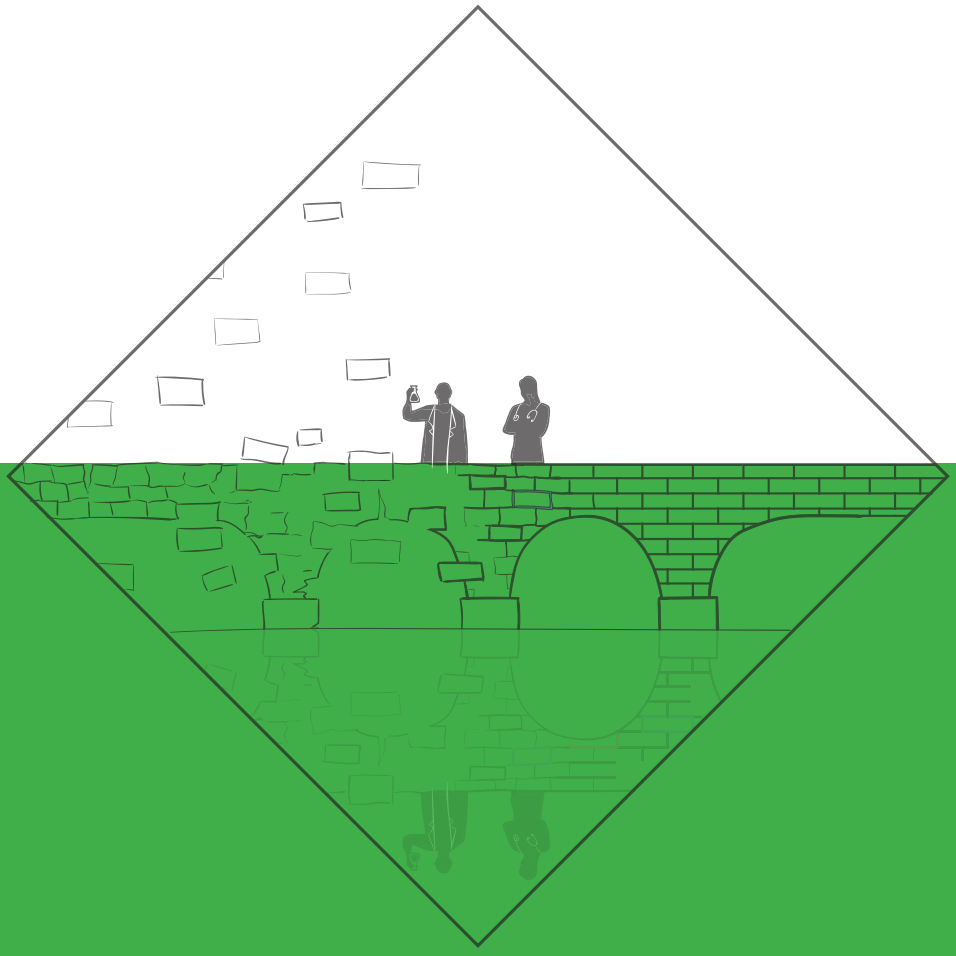
General introduction



Chapter 1

General introduction

Cancer is a complex genetic disease that arises after accumulation of mutagenic events. Alterations in oncogenes, potential cancer-initiating genes, in combination with loss of tumor suppressor genes lead to a tumorigenic cellular state, in which the balance between proliferation and cell killing signals is heavily skewed towards the former. Since genetic alterations drive tumorigenesis, genomic instability can facilitate this process, and progressively enables natural selection of cancer cells that are better equipped to survive, proliferate, evade the immune system and metastasize. Over the past decades, many common hallmarks of cancers have been described that contribute to tumorigenesis (1).

For a subset of cancers, single oncogenic events have been identified as the driving force that those cancers heavily depend on for their proliferation and survival, also called oncogene addiction. Many oncogenes operate in growth factor pathways either upstream at a receptor level (Epidermal Growth Factor Receptor (EGFR), at intracellular signaling level (BRAF, RAS, PI3K), or at the downstream effector level (transcription factors e.g. Myc). For instance, activation mutations in, or overexpression of, EGFR family proteins is observed in many cancer types, affecting cancer hallmarks such as cell proliferation and metastasis (2). Oncogene addiction has formed the rationale to develop molecularly targeted cancer therapies against actionable oncogenes. These therapies mainly consist of compounds targeting oncogenic kinases to block their functionality, or compounds that prevent target activation, e.g. through prevention of ligand – receptor interaction. Ultimately, targeting of the oncogene is aimed to switch from a tumorigenic and 'pro-survival' state into a 'pro-apoptosis' state (3).

Besides the identification of strong oncogenic drivers, tumors depend on supportive cellular processes that are essential to maintaining a tumorigenic state, but without being sufficient to drive or initiate oncogenic transformation (4). For example, deleterious mutations in the homologous recombination DNA repair mechanism leads to highly mutagenic tumors, however these tumors are highly sensitive to additional DNA damage induced by chemotherapy and are highly depended on other DNA repair mechanisms. Such non-oncogene addiction offers new strategies for therapeutic intervention and take advantage of the higher dependency of tumor cells on common cellular processes such as metabolism, mitosis, DNA replication and repair (5).

Both oncogene-addiction and non-oncogene addiction can form the basis of cancer therapies, either as a single agent or used in combination treatments. A frequent problem with targeted agents is the acquired resistance that tumors frequently develop. Treatment resistance can develop through selective pressure on pre-existing or acquired secondary DNA mutations that nullify the treatment response. Alternatively, treatment resistance can arise through molecular rewiring of signaling pathways, hindering their effectiveness. For example, pre-clinical studies have shown that inhibition of HER2 or downstream AKT kinases can induce novel expression of HER3, leading to rewiring of downstream signaling and reconstitution of oncogenic drive (6,7). Understanding the mechanisms behind, and more importantly, timely determination of treatment resistance is therefore highly relevant for clinical practice.

In order to measure treatment efficacy and rewiring of cellular processes induced by cancer treatments, in this thesis we used proteomic analysis to capture the dynamics of both transcriptional and translational processes. In particular, we used stable isotope labeling of amino acids (SILAC) to metabolically label cell cultures to such an extent that isotope-labeled proteins can be distinguished from unlabeled proteins due to mass differences using liquid chromatography mass spectrometry (LC-MS) (8). This enables mixing of cell lysates from labeled

and unlabeled cell cultures to measure protein expression changes induced by perturbations to either cell culture in a single MS analysis to greatly increase the accuracy of quantification. Using SILAC, proteome-wide responses to therapeutic targeting of oncogenic and non-oncogenic processes can be measured to gain insight into the wiring and rewiring of those processes, and to possibly discover novel markers of therapy response (effect sensors).

Aim of the thesis

To determine at the proteomic level how cancer cells deal with therapeutic targeting of oncogenic pathways or supporting non-oncogenic pathways to identify effect sensors and uncover mechanism of treatment efficacy.

In order to address this aim, we set out to use quantitative analysis of cancer proteomes to answer two primary questions:

1) Can we discover robust proteomic markers for cancer treatments, and can we use those to monitor treatment response?

2) Can proteomic analysis of the cell cycle provide insights into regulation of DNA damage responses?

Outline of the thesis

Using the EGFR family as model, we discuss in **chapter 2** how molecular imaging may be employed to characterize the target expression of HER family members in tumor lesions, monitor drug effectiveness and emergence of treatment resistance. We further exemplified how genomic and proteomic analysis can guide selection of 'effect sensors'; new molecular imaging targets, which are critical and informative markers for treatment effectiveness or resistance.

In **chapter 3**, we set out to identify effect sensors for EGFR-targeted agents. Using SILAC proteomics, we investigated the cellular rewiring at the proteomic level in response to EGFR inhibition in breast cancer cell lines. We focused on identifying proteins that are present at the plasma membrane upon drug treatment, as these would be the ideal targets for molecular imaging. This effort resulted in the identification of Mucin-1 (MUC1) to be specifically upregulated in response to treatment with a variety of EGFR-targeted agents. The underlying mechanistic processes were explored, and whether modulation of MUC1 can be assessed in vivo by molecular imaging.

The proteomic response of cancer cells to drugs is influenced greatly by both tissue origin and the mutational background of cancer cells. Furthermore, physiological coping mechanisms to cellular stress may underlie cellular responses to anti-cancer drugs. Therefore, in **chapter 4**, we measured changes in protein abundance induced by EGFR-targeting and DNA-damaging drugs in breast, lung, and colon cancer cell lines using SILAC-MS. Using the resulting datasets, we analyzed generic responses versus drug-specific, as well as tissue-specific responses, to identify potential effect sensors for various cancer therapies.

Besides the use of SILAC proteomics in identification of biomarkers for cancer, it is highly valuable in elucidating biological processes. In **chapters 5-7**, the intricate process of cell division and proper separation of DNA to daughter cells was studied in the context of DNA damaging therapeutics. The onset, transition and exit from mitosis (M-phase) to new daughter cells (G1-phase) is a highly regulated process, which is -in part- regulated by the E3 ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C). In **chapter 5**, we reviewed how the APC/C controls the exit of mitosis by targeting multiple mitotic regulators for proteasomal degradation. In addition, we described how this E3 ligase is under control of DNA damage-induced signal transduction, and how the APC/C controls cell fate in situations of genotoxic stress.

Next, we used an integrated proteomics and bioinformatics approach to identify

proteins regulated by APC/C. From this analysis, we identified the DNA damage repair protein C-terminal binding protein 1 interacting protein (CtIP), a DNA end-resection factor in homologous recombination (HR), and Rap1-interacting factor (Rif1), a regulator of non-homologous end joining (NHEJ), to be degraded after mitosis. In **chapter 6,** we described how CtIP is recognized by APC/CCdh1 and how APC/CCdh1-mediated control of the abundance of CtIP contributes to DNA damage repair and genome maintenance.

In **chapter 7**, we further studied how DNA damage response proteins are rewired during mitosis, and identified a novel role for Rif1. Instead of a role in NHEJ, we discovered that Rif1 is involved in the resolution of DNA intermediates that are visible as ultrafine DNA bridges (UFBs) in anaphase. We studied how Rif1 interacts with known UFBs localizing proteins and studied the consequences on DNA segregation when UFB-resolving factors are defective.

Finally, in **chapter 8**, our findings are summarized and discussed in terms of mechanistic implications, impact on cancer treatment, and future studies.

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