Emerging opportunities to target gene transcription and DNA repair in drug discovery

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CHAPTER 1

Introduction and Scope of the Thesis

INTRODUCTION

After the completion of the Human Genome Project, biological research in the post-genomic era has focused on interpreting the biological functions of genes and their products. This includes investigating the regulation of DNA transcription as well as DNA replication and damage repair. These aspects are intricately connected to regulated cell death and diseases associated with it, such as drug-resistant cancers and neurodegenerative diseases. The discovery of novel DNA processing mechanisms that modulate cell death, whether by inducing or inhibiting it, could unveil promising therapeutic strategies for treating diseases related to cell death. DNA processing has several aspects, including DNA transcription, DNA replication, and DNA damage repair. This thesis mainly focuses on the modulation of DNA transcription by histone lysine acetylation and on DNA damage repair mechanisms. The aim of this thesis is to elucidate the regulatory mechanisms of DNA processing involved in regulated cell death and their role in disease pathogenesis, meanwhile developing novel small molecular entities to treat cell-death-related diseases based on the mechanisms identified in this thesis.

1. Histone Lysine Acetylation

Acetylation is one of the main post-translational modifications (PTMs) that form the histone code, which is widely involved in the regulation of DNA transcription. Acetylation of lysine residues on the histone tails is directly involved in the regulation of gene transcription. Already in 1964, it was found that histone binding to DNA inhibits gene transcription and that chemical acetylation of histones reduces this inhibitory effect[1]. The activation of gene transcription upon histone acetylation can be explained by a reduction of the charge-charge interactions between DNA and histones, which provides a less tightly packed chromatin structure. This allows transcription factors to bind to the DNA, thus promoting gene transcription.

Apart from regulating DNA-histone interaction histone acetylation also regulates protein-protein interactions, such as histone-bromodomain interactions. Bromodomains are protein interaction domains that recognize ε-*N*-lysine acetylation. Proteins containing bromodomains are mostly transcription factors and transcriptional regulators (e.g., the BRD and BAZ family). These proteins anchor to acetylated lysine tails to get close to the DNA. Additionally, several histone modulators (histone methyl- and acetyltransferases) contain bromodomains, suggesting the existence of cross-talk mechanisms in which acetylation of the histone lysine residues precedes subsequent post-translational modification of the histone as a mechanism to write the "histone code"[2].

Acetylation levels of lysine residues are regulated by a dynamic equilibrium of acetylation and deacetylation reactions. Whereas, histone acetyltransferases (HATs) catalyze lysine acetylation reactions, histone deacetylases (HDACs) catalyze hydrolysis of acetylated lysine residues. The balance between acetylation and deacetylation reactions is disturbed in many diseases, such as cancers and inflammatory diseases.

1.1 Histone Acetyltransferases

HATs acetylate histones by transferring an acetyl group from acetyl coenzyme A to ε-amino groups of lysine residues on histone tails[1]. HATs acetylate not only histones but also several non-histone proteins as well[2]. Until now, several families of HATs consisting of more than 20 subtypes have been discovered in plants, animals, and

fungi[3].

HATs are classified into type-A and type-B based on their cellular locations. Most of the HATs are nuclear enzymes (type-A), which acetylate histones that are attached to DNA[4]. The main functions of type-A HATs are to maintain the epigenetic 'histone code' in newly-divided cells and to regulate histone acetylation level in responses to cell signaling. Type-B HATs, also denoted as cytoplasmic HATs, are found in both the cytoplasm and in the nucleus[4]. Cytoplasmic HATs modify free histones in the cytoplasm just after their synthesis. Nuclear HATs are transcriptional regulators involved in a variety of cellular processes like cell cycle control, differentiation, DNA damage repair, and apoptosis. HATs also frequently form complexes with other proteins. Such complexes have distinct functions, such as increasing the specificity of HATs to histone acetylation, targeting specific genes, as well as expanding the range of targets to non-histone proteins.

1.2 Bromodomain

Bromodomains are a type of protein domains that recognize and bind to acetylated lysine residues on histones[3]. Bromodomains are found in a variety of proteins that are involved in the regulation of gene expression, chromatin remodeling, and other cellular processes[4]. The conserved four-helix bundle fold is the core structure of a bromodomain, which forms a hydrophobic pocket that specifically recognizes and binds to acetylated lysine residues[5]. The binding of bromodomains to acetylated lysine residues is a key step in the recruitment of protein complexes to chromatin, ultimately leading to gene expression changes. Due to their essential roles in chromatin regulation and gene expression, bromodomains have emerged as promising targets for drug development in various diseases, including cancer and inflammation.

2. DNA Damage Repair Mechanisms

DNA damage repair is an essential aspect of DNA processing to maintain the proper function of the genome. Cells have evolved a number of highly efficient DNA repair pathways that counteract double-strand breaks, such as homologous recombination (HR) and non-homology end joining (NHEJ). HR is a precise repair process that plays a crucial role in maintaining genome stability. The process begins with the recognition and resection of damaged DNA ends, followed by invasion of a homologous DNA

sequence into the damaged site. Later, DNA synthesis and branch migration occur, resulting in the formation of a Holliday junction. After resolving the Holliday junction, the original DNA sequence will be restored. By contrast, NHEJ is an error-prone process. NHEJ fixes double-strand breaks by directly ligating the broken ends without requiring a homologous template, which can result in small insertions or deletions at the repaired site, leading to loss of gene function. Nucleases are vital for both pathways. Investigating the involvement of other nucleases, such as macrophage migration inhibitory factor (MIF), in these genetic processes provides valuable insights. It enhances our understanding of DNA repair, and genome stability, uncovering new mechanisms and potential therapeutic targets.

2.1 Macrophage Migration Inhibitory Factor

In 1966, MIF was initially identified as a lymphokine, derived from activated T-cells, that inhibited the random migration of macrophages[6]. Currently, we know MIF as a widely secreted pleiotropic cytokine that is involved in multiple processes[7–9]. MIF has a trimeric structure in which each monomer has a molecular weight of 12.5 kDa and consists of 115 residues arranged in two α-helices packed against four *β*-strands forming a central barrel in the active homotrimer[10]. One aspect of MIF family proteins that remains enigmatic is the presence of catalytic sites such as the tautomerase active site that requires the *N*-terminal proline[11], and the oxidoreductase active site that requires Cys56 and Cys59[12–14]. Although no physiological substrate has been identified for the MIF tautomerase active site, the “pseudosubstrates” phenylpyruvate (PP) or 4-hydroxyphenylpyruvate (4-HPP) proved to be suitable to screen MIF binders that influence MIF tautomerase activity[14,15]. Furthermore, MIF has been shown to harbor both 3′ exonuclease and endonuclease activity independent of its oxidoreductase and tautomerase activities[16]. Moreover, MIF is known to be involved in protein-protein interactions. The hydrophobic surface area of MIF has 4 potential binding sites that stand out due to their hydrophobic contact agglomeration available for interactions with proteins and small molecules (Chapter 6, Figure.1a). These interaction sites enable, for example, binding to and activation of membrane receptors, such as the cluster of differentiation 74 (CD74)[17] and C-X-C motif chemokine receptors 2[18,19], 4[19], and 7[20] (CXCR2, 4, and 7). Protein-protein interactions also facilitate intracellular MIF activities in cell signaling and gene

transcription. For example, MIF interacts with thioredoxin (TRX) to induce nuclear factor kappa light chain enhancer of activated B cell (NF-κB)-mediated signaling[21]. Furthermore, MIF forms a complex with p53 to attenuate p53-mediated gene transcription[22] and coordinate the cell cycle with DNA damage checkpoints[23]. In 2016, MIF was identified as a crucial executor in parthanatos by the formation of a MIF/apoptosis-inducing factor (AIF) complex that translocates from the cytosol to the nucleus triggering DNA fragmentation and cell death. Overall, the role of MIF in protein-protein interactions in various disease models[24,25] is clear. The development of small molecule modulators of MIF protein-protein interactions holds promise for chemical-biological investigation of MIF function as well as drug discovery.

3. Regulated cell death

Maintenance of proper DNA transcription and DNA repair processes are essential to cell survival. Failure of DNA repair may activate several types of regulated cell death. Regulated cell death is a vital biological process that maintains tissue homeostasis and eliminates damaged or unwanted cells. It plays a crucial role in various physiological and pathological conditions. There are several types of regulated cell death, such as apoptosis, autophagy, necroptosis, parthanatos, and ferroptosis, each with distinct molecular mechanisms. Apoptosis is a highly controlled process involving cellular shrinking, chromatin condensation, and fragmentation. Autophagy involves the degradation of cellular components to recycle nutrients. Necroptosis is a programmed form of necrosis, characterized by cell swelling and membrane rupture. Parthanatos is a form of caspase-independent regulated cell death resulting from the accumulation of poly (ADP-ribose) (PAR) polymers. Ferroptosis is a type of regulated cell death marked by iron-dependent lipid peroxidation. Regulated cell death is tightly regulated by a complex interplay of signaling pathways in which DNA transcription and DNA repair processes are widely involved. This thesis mainly focuses on apoptosis, parthanatos, and ferroptosis.

3.1 Apoptosis

Apoptosis is a natural process of programmed cell death that occurs in cells as part of normal physiological processes[33]. It is characterized by a series of biochemical and morphological changes that ultimately lead to the death of the cell. Apoptosis is vital

in maintaining tissue homeostasis and eliminating damaged or no longer needed cells.

In cancer treatment, apoptosis is often targeted to induce cell death in cancer cells. One promising approach is the use of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)[34]. TRAIL is a protein that selectively induces apoptosis in cancer cells while sparing normal cells. It works by binding to death receptors on the surface of cancer cells, triggering a signaling cascade that ultimately leads to cell death[35].

TRAIL-based therapies have shown promise in preclinical studies as a potential cancer treatment. However, there are still challenges to overcome, including the development of resistance to TRAIL-induced apoptosis and the need to identify biomarkers to predict which patients are most likely to respond to TRAIL-based therapies[36].

3.2 Parthanatos

Parthanatos is a form of caspase-independent regulated cell death resulting from the accumulation of poly (ADP-ribose) (PAR) polymers and characterized by a unique pathway, distinct from apoptosis, necroptosis, and any other types of cell death. Parthanatos is involved in a wide range of diseases, such as ischemic stroke[26], glutamate excitotoxicity[27], inflammation[28], reactive oxygen species (ROS)-related damage[29], and neurodegenerative diseases[30]. The term parthanatos was created after *Thanatos*, the personification of death in Greek mythology, to refer to PAR-mediated cell death[31]. The parthanatos cascade involves PAR polymerase 1 (PARP-1) overactivation, PAR accumulation, PAR binding to the death effector apoptosis-inducing factor (AIF), AIF release from the mitochondria and its nuclear translocation[32]. AIF is a mitochondrial oxidoreductase that participates in the biogenesis of the respiratory chain in physiological conditions. In parthanatos, AIF induces chromatin condensation and DNA fragmentation. However, the mechanism of how AIF induces DNA fragmentation was unknown until MIF was identified as a nuclease in 2016. Upon activation of parthanatos, AIF recruits MIF to form a complex that translocates from the cytosol to the nucleus to trigger DNA fragmentation and cell death. Overall, the discovery of a role for the MIF/AIF interaction in PARP-1-mediated cell death suggests a potential for small molecule modulation of this interaction.

3.3 Ferroptosis

Ferroptosis is a type of regulated cell death[37] that has gained significant attention in recent years due to its role in a number of human diseases, such as neurodegenerative diseases, organ injury, and cancers[38,39]. A key process in ferroptosis is the iron(II) catalyzed non-enzymatic Fenton reaction to provide lipid peroxides. It is commonly perceived that high levels of lipid peroxides cause membrane rupture and subsequent cell death[40]. Ferroptosis is distinct from other forms of regulated cell death (RCD), such as parthanatos, apoptosis, and necroptosis[37] and it does not seem to be sensitive to the development of resistance to chemotherapy[41]. Therefore, ferroptosis induction by inhibition of ferroptosis protection systems holds promise to overcome therapy resistance cancer. To date, four major ferroptosis defense systems have been elucidated. Firstly, Glutathione peroxidase 4 (GPX4) utilizes glutathione (GSH) to reduce excessive lipid peroxidation[37,42]. Secondly, Ferroptosis suppressor protein 1 (FPS1) suppresses ferroptosis by generating two natural antioxidants, ubiquinol (CoQQH2) and Vitamin K hydroquinone (VKH_2)[43–45]. Thirdly, GTP cyclohydrolase 1 (GCH1) controls lipid peroxidation via its metabolic antioxidant, tetrahydrobiopterin (BH_4)[46]. Fourthly, dihydroorotate dehydrogenase (DHODH) suppresses mitochondrial ferroptosis by reducing ubiquinone to ubiquinol[47]. These proteins have successfully been exploited for the development of small-molecule ferroptosis inducers. Nevertheless, full exploitation of the potential of ferroptosis in induction needs a further understanding of the molecular mechanisms involved.

This thesis mainly focuses on the modulation of DNA transcription by histone lysine acetylation and on DNA damage repair mechanisms. The aim of this thesis is to elucidate the regulatory mechanisms of DNA processing involved in regulated cell death and their role in disease pathogenesis, meanwhile developing novel small molecular entities to treat cell-death-related diseases based on the mechanisms identified in this thesis. Taken together, molecular mechanisms involved in the modulation of DNA transcription and DNA damage repair hold promise to modulate distinct mechanism of regulated cell death that hold promise for drug discovery.

SCOPE OF THIS THESIS

This thesis focuses on discovering mechanisms that control cell death and unveils promising potential therapeutic strategies for treating cell death-related diseases with small molecular compounds.

In **Chapter 2**, we summarized the current knowledge about experimental approaches toward development of small molecule modulators of histone acetyltransferase activity as therapeutics in various diseases. Meanwhile, we describe the prospects for future development of such modulators.

In **Chapter 3**, we applied the P300/CBP selective HAT inhibitor, A485, to sensitize non-small-cell lung carcinoma to TRAIL treatment. This study highlighted the aberrant regulation of histone acetylation, that is widely found in cancers and promotes resistance towards TRAIL-based therapies.

In **Chapter 4**, we generated an activity-based probe (**Labelox B**) for activity-based labelling of lipoxygenase (LOX) family enzymes in one-step. **Labelox B** enables visualization of active LOXs in cells in addition to antibody-based total LOXs detection. Moreover, we found that lipoxygenases are also active in specific localizations in the nucleus, which is supported by identifying a bromodomain-like region in 15-LOX-1 that directly interacts with acetylated histone H3. The presence of an epigenetic reader domain in 15-LOX-1 could also play a role in epigenetics and chromatin remodeling.

In **Chapter 5**, we use 4-iodopyrimidine (4-IPP) as a covalent binder of MIF and D-dopachrome tautomerase (DDT) to generate probes to label MIF family proteins. 4-IPP-derived probes potently labelled MIF and DDT in cells, thus enabling tracking MIF translocating (this Chapter) and MIF internalization in living cells (**Chapter 7**). This demonstrates that 4-IPP-derived probes can be employed as chemical tools for to investigate the biological function of MIF family proteins.

In **Chapter 6**, we developed a class of allosteric inhibitors of MIF which interfere with recruitment of MIF to AIF and MIF nuclear translocating, which is a process that is crucial for parthanatos. As such, these allosteric MIF inhibitors demonstrate a conceptual potential for treatment of neurodegenerative diseases.

In **Chapter 7**, we demonstrated that MIF plays a role in protection against ferroptosis induction. We attributed this protective effect to a role in DNA repair by homologous recombination (HR). Thus we could for the first time establish a connection between HR and protection against ferroptosis induction. Moreover, we could identify MIF as a protein that is crucial for HR-mediated protection against ferroptosis. These insights complement the current knowledge on HR. Moreover, the MIF functions in ferroptosis proved to be connected to proteins such as BRCA1, and RAD51, which indicates a mechanistic link between MIF and these intensively exploited drug targets in oncology.

In **Chapter 8**, we summarize the work described in this thesis and provide prospects for future studies.

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