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Thesis title: Regulation of Human Heterochromatin Protein1 Binding Protein 3 (HP1BP3, also known as HP1BP74) in response to DNA Damage.

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Abstract

DNA damage can result from exogenous agents such as exposure to ionizing radiation (IR), ultraviolet radiation (UV), environmental toxins or drugs or from endogenous sources such as reactive oxygen species or errors during DNA replication. The DNA repair involves both post-translational modification of nucleosomes and concentration of DNA repair proteins at the damaged site(s). Therefore, the nucleosome rearrangement and chromatin disassembly with histones and other non-histone proteins upon damage must be in a dynamic balance with chromatin reestablishment followed repair that may otherwise cause extensive damage and trigger the growth of tumours. However, most of studies mainly focused on the core histones, whereas the heterochromatin proteins and their partners' identity remained unknown.

Recent studies in Dr Nateri's laboratory identified the heterochromatin protein HP1 binding protein 3 (HP1B3, also known HP1-BP74) as one of the FBXW7 (F-box and WD repeat domain containing 7, E3 ubiquitin protein ligase, also known Fbw7, CDC4, sel10) interacting protein, using a modified yeast 2-hybrid reverse Ras-recruitment system (rRRS).

HP1BP3 is a predicted nuclear protein with 553 amino acids with distinct protein interaction domains. When I started working at Dr Nateri's laboratory, apart from a single publication (Hayashihara et al., 2010) very little was known about the HP1BP3 protein. HP1BP3 is a chromosomal protein that exists as a component of heterochromatin and is involved in chromatin function and structure. Our laboratory recent analysis also indicate that HP1BP3 degradation by the E3 ubiquitin ligase Fbxw7 regulates of mouse hematopoietic stem cell (HSC) cycle entry and the transcriptional "signature" that is associated with the quiescent, self-renewing of HSC phenotype and leukemic status (Abuzinadah et al., submitted). To this end, our laboratory and more recent data from other groups suggest that the regulation of HP1BP3 may determine a key role in many types of biological events occurred in normal and cancer cells.

To investigate the molecular mechanisms underpinning HP1BP3 protein activity regulation that signal into biological response, we initially investigated structural features and phosphorylation sites of the HP1BP3 protein using of various web-based computational and bioinformatics analyses. We determined the distribution of several clusters of amino acid residues surrounding the DNA damage-regulated phospho-SQ (S: Ser & Q: Gln), a consensus ATM and ATR phosphorylation motif on the HP1BP3, while this is consistent with the notion that E (Glu) or D (Asp) is often found around SQ or TQ sequences phosphorylated by ATM or ATR kinases in vitro. We have therefore hypothesized that HP1BP3 protein could be a potential target of ATM/ATR in cells treated with DNA-damaging agents.

Herein, we present our experimental evidences, build on a wide range of cell and molecular biology tools and biochemical approaches to analyze the HP1BP3 regulation in response to DNA-damaging agents, and conclude a possible cross talk between HP1BP3 protein and activation of ATM and possibly ATR kinases via:

1) Identification of nuclear localization signals within the HP1BP3 protein (chapter 3)

2) ATM/ATR kinases coordinate the expression of HP1BP3 protein level and phosphorylation in response to DNA-damaging agents, such as UV light, ionizing radiation and chemotherapeutic drug in human fibroblasts (chapter 4)

3) FBXW7-mediated degradation of HP1BP3 protein is antagonized by ATM/ATR activation in human colorectal cancer (CRC) cells (chapter 5)

4) Knockout of HP1BP3 generated using CRISPR/Cas9 system, caused p53 accumulation in HCT116 CRC cells lacking of FBXW7 after exposure to ionizing radiation (chapter 6).

From these data we also concluded that further research is required to explore biological significance of HP1BP3 phosphorylation events modulated by ATM/ATR, to study mechanistic

changes occurred on patterns of histone modifications as a result of HP1BP3 phosphorylation and the influence of nucleosome packing degree on the integration of DNA repair signaling and the complexity of the chromatin architecture. In addition, another challenge in our laboratory is to identify the direct target genes that are regulated via HP1BP3/ATM signaling axis, and to show how they contribute to cancer development, which could be critical for designing therapies to compensate mutation(s) of upstream regulator such as FBXW7 tumour suppressor gene in human cancers.