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Faculty of Veterinary Medicine

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Cell Apoptosis and Its Relation to Cancer Treatment

A thesis presented

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Ph.D.

(Cytology & Histology)

Under supervision of

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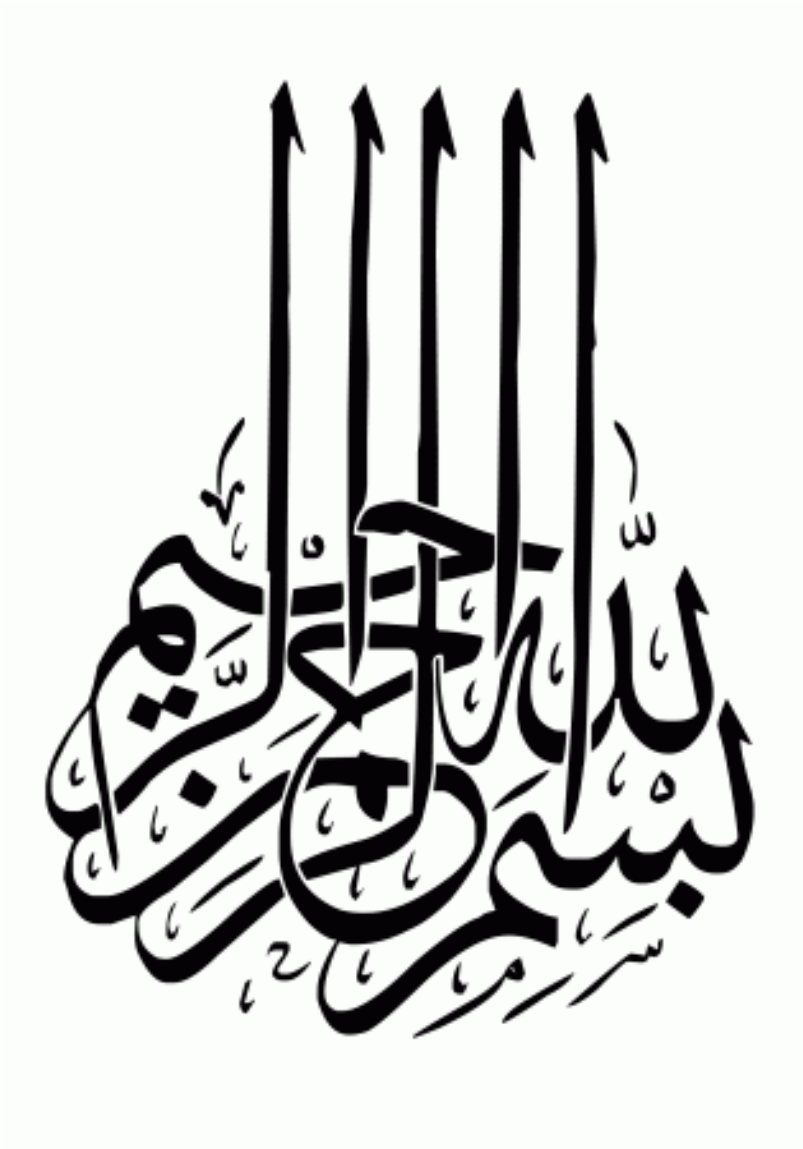
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APPROVAL SHEET

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ABSTRACT

Apoptosis is one of programmed cell death pathways, it is a physiological process through which the animal could organize the number of cells in tissues. Failure of apoptosis results in different diseases including cancer. Prostate cancer remains the second leading cause of cancer related death in men. Therefore, induction of apoptosis has become one of the most effective strategies for cancer treatment. In our study, we induced apoptosis via targeting the ubiquitin proteasome system (UPS). Inhibition of UPS was achieved by using proteasome inhibitors mainly natural 19S inhibitors as isothiocyanates (ITCs) which found abundantly in cruciferous vegetables. We hypothesize that ITCs as electrophiles could interact with the catalytic triads (CYS, HIS and ASP) of the 19S associated USP14 and UCHL5, ultimately inhibiting their activities. Docking and biochemical results suggest that ITCs are potent inhibitors of UCHL5 than USP14. Indeed, Ub-VS assay confirmed the inhibitory activity of each ITC as the ubiquitin binding activity of UCHL5 and USP14. This inhibition of USP14 and UCHL5 caused increased levels of USP14 and UCHL5 proteins but not the third 19S DUB, RPN11 suggesting feedback loop activation and further supporting that ITCs are inhibitors of proteasomal cysteine DUBs. Also, DUBs inhibition was associated with significant accumulation of ubiquitinated proteins, induction of apoptosis, inhibition of cells proliferation, suppression of cell invasion and degradation of androgen receptor which is considered an important driver of castration resistance prostate cancer (CRPC). Curcumin treatment induced apoptosis and downregulation of androgen receptor in a dose and time dependent manner. Chemical inhibitors of proteasome exhibited antiproliferative effect and induced apoptosis in a dose and time dependent manner. Bortezomib showed a synergetic effect with b-AP15 in induction of apoptosis and downregulation of androgen receptor variant 7. Knocking down of USP14 or UCHL5 increased the sensitivity of the knocked down cells to the anticancer therapy. Our finding of ITCs as proteasomal cysteine DUB inhibitors should provide insightful information for designing, discovering and developing potent, specific 19S-DUB inhibitors for cancer therapies.

Key words:

Apoptosis, Ubiquitin proteasome system, Deubiquitinating enzymes, USP14, UCHL5, Isothiocyanate, BITC, PEITC, SFN, Curcumin, Androgen receptor, Castration resistant prostate cancer.

DEDICATION

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Introduction

Apoptosis is one of programmed cell death pathways **(Ouyang *et al.*, 2012)**, it is a physiological process through which organisms could regulate the number of cells in tissues **(Ellis *et al.*, 1991)**. Failure of apoptosis results in many diseases including cancer due to the uncontrolled proliferation of cells that leads to tumor growth **(Hoeppner *et al.*, 1996 and Evan and Vousden, 2001).** Therefore, one of the most important strategies in cancer treatment is induction of apoptosis **(Hong and Sporn, 1997; Jaffrézou *et al.*, 1998; Debatin, 2000; Kelloff *et al.*, 2000 and Woynarowska and Woynarowski, 2002).** Induction of apoptosis could be through targeting the ubiquitin proteasome system (UPS) which controls different cellular processes and is considered the major regulator of protein degradation in eukaryotes **(Hershko and Ciechanover, 1998; Goldberg, 2007; Finley, 2009; Schrader *et al.*, 2009 and D'Arcy and Linder, 2012).**

The ubiquitin proteasome system (UPS) is composed of a small molecule of ubiquitin and large multiunit complex called 26S proteasome, ubiquitin molecule is responsible for tagging of the protein substrate to the 26S proteasome for its degradation. The 26S proteasome is composed of 20S barrel shaped structure which is responsible for the proteolytic activity of the proteasome capped on each end by one or two 19S regulatory particles **(Hershko *et al.*, 1983; Groll *et al.*, 1997; Amerik and Hochstrasser, 2004; Goldberg, 2007; Lee *et al.*, 2010; D'Arcy and Linder, 2012 and Nguyen *et al.*, 2013).** Ubiquitination is a reversible process that includes ubiquitin

ligating (E3 ligase) and could be reversed by deubiquitination through deubiquitinating enzymes (DUBs) **(Liao *et al.*, 2017).** Deubiquitinating enzymes (DUBs) are responsible for removal of ubiquitin or ubiquitin like chain from the target protein before its degradation in the 20S proteasome in eukaryotes **(Song and Rape, 2008 and Aressy *et al.*, 2010).**

There are approximately 100 DUBs encoded by human genome **(Liao *et al.*, 2017)**, three of them are associated with the 19S proteasome in the mammalian cells **(Lee *et al.*, 2011 and D'Arcy *et al.*, 2015).** Ubiquitin specific protease 14 (USP14) and Ubiquitin carboxyl-terminal hydrolase isozyme L5 (UCHL5) belong to cysteine proteases class while the third one is proteasome regulatory particle lid subunit RPN11 (RPN11) and it belongs to the metalloprotease class (**Lee *et al.*, 2011; D'Arcy *et al.*, 2015 and Wang and Linder, 2015)**. These proteasome's associated DUBs play an important role in the maintenance of ubiquitin homeostasis **(Lee *et al.*, 2011 and D'Arcy *et al.*, 2015).**

Since tumor tissues show overexpression of different DUBs **(Daviet and Colland, 2008; Wang *et al.*, 2016 and Liao *et al.*, 2017),** DUBs could be an important target for treatment of different cancers including prostate cancer which is considered the second leading cause of cancer related death in men **(Jemal *et al.*, 2007; Knudsen and Kelly, 2011 and Lu and Luo, 2013).** Also, DUBs are associated with the stabilization, co-regulation and transcription of androgen receptor (AR) **(Liao *et al.*, 2017)** which is considered the fundamental driver

for the progression of castration resistant prostate cancer (CRPC) **(Tamura *et al.*, 2007; Chen *et al.*, 2008 and Attard *et al.*, 2009).** Therefore, inhibition of these proteasome associated DUBs, or their silence may be a promising strategy for downregulation of androgen receptor (AR) and induction of apoptosis in prostate cancer cells. The aim of this study is to obtain a potent natural or chemical compound that could inhibit the development of cancer, particularly prostate cancer and breast cancer which remain the second leading cause of cancer related death in men and women, respectively. In our study, we used different proteasome inhibitors including chemical inhibitors and natural compounds which present in cruciferous vegetables as broccoli, these natural compounds include isothiocyanates such as benzyl isothiocyanate (BITC), phenethyl isothiocyanate (PEITC) and sulforaphane (SFN) in addition to curcumin which was extracted from *curcuma longa* plant. We tested the inhibitory activity of these compounds on DUBs and observed the antiproliferative activity of these compounds on cell growth in addition to the relation between DUBs inhibition or silence and the expression of androgen receptor, particularly androgen receptor variant 7 (AR-V7) which has an important role in the progression of Castration resistant prostate cancer (CRPC). The present study should provide insightful information for designing, discovering and developing potent, specific 19S-DUB inhibitors for cancer therapies.