BIOINFORMATICS ANALYSIS OF THE STRUCTURAL AND BIOCHEMICAL EFFECT OF THE CHEMICAL WEAPON MUSTARD GAS ON THE P53 PROTEIN AND CELL DEATH

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This dissertation is dedicated to:

My decedent Mother

My father

My brother and sister

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ABSTRACT

The universal and extensive usage of Sulfur Mustard gas (SM) as a disqualification chemical warfare potential in the past century has demonstrated its long-term toxic impacts. Sulfur mustard (SM), also known as mustard gas, is an alkylating compound used as a chemical weapon in World War I and by Iraqi forces against Iranians and indigenous Iraqi Kurds during the Iran–Iraq War of the 1980s. We can notice the carcinogenic effects of exposure to SM gas. The present study characterizes Bioinformatics analysis of the structural and biochemical effect of the chemical weapon mustard gas on the P53 protein and cell death. Inactivation of the p53 gene is essentially due to small mutations (missense and nonsense mutations or insertions/deletions of several nucleotides. For this study we used from Primary databases (experimental results directly into database), secondary databases (results of analysis of primary databases) and aggregate of many databases (Links to other data items, combination of data and consolidation of data).The database used includes: IARC TP53, P53web site-free, UniProt, NCBI and PDB format. Also, we used from some software in methodology such as PYMOL and CROMACS for visualizing, molecular dynamics simulation and analysis. We assay the most important of P53 region means that DNA-binding domain(DBD) from the point of view protein stability after mutation and the its effects on cell cycle arrest (cell death).The results have shown that all missense mutations selected in this case had caused remarkable flexibility and stability on DBD of the P53.Structural alterations had not been observed in DNA-binding domain, so it may be through functional changes in the certain amino acid residues and bounding linkage to DNA.

ABSTRAK

Penggunaan berleluasa sulfur mustard sebagai agen kimia berbahaya telah terbukti membawa kesan tosik yang berpanjangan. Sulfur mustard (SM) juga dikenali sebagai gas mustar, merupakan bahan beralkali yang digunakan sebagai senjata di Perang Dunia I dan pihak tentera Iraq dalam menentang pihak Iran juga puak-puak Iraq Kurdis semasa perang Iran-Iraq dalam tahun 1980. Pendedahan SM membawa kesan karsinogenik. Kajian semasa mengklasifikasikan analisa bioinformatik ke atas struktur dan kesan biokimia senjata gas mustard terhadap protein p53 dan sel mati. Penyahaktifan gen p53 secara asasnya disebabkan oleh mutasi gen (mutasi tidak logik atau penambahan/pengurangan beberapa nukleotida).Pangkalan data utama (keputusan eksperimen yang langsung dimasukkan ke dalam pangkalan data), Pangkalan data sekunder (analisa keputusan dari pangkalan data utama) dan agregat pangkalan data (menghubungkan ke data lain,kombinasi data dan konsolidasi data) telah digunakan dalam kajian ini. Pangkalan data lain yang digunakan termasuklah: IRAC TP53, P53 laman sesawang, UniProt, NCBI dan format PDB. Selain itu, perisian PYMOL dan CROMACS juga digunakan bagi tujuan visualisasi, simulasi dinamik molekul dan analisis. Kami assay yang paling penting P53region bermakna bahawa DNA mengikat domain(DBD) dari sudut pandangan protein kestabilan selepas mutasi dan kesannya terhadap kitaran menangkap sel (sel mati). Keputusan telah menunjukkan bahawa semua mutasi missense dipilih dalam kes ini telah menyebabkan fleksibiliti yang luar biasa dan kestabilan di DBD daripada P53 yang. Perubahan struktur itu tidak dipelihara dalam DBD, jadi ia mungkin melalui perubahan berfungsi dalam sisa-sisa asid amino tertentu dan Mengehad hubungan untuk DNA.

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Today's civilized world, in spite of international treaties and agreement that have banned the utilization and accumulation of chemical weapons, some countries continue to hold a significant amount of stockpile as part of their legacy arsenal or for deterrent purposes. The relative ease to manufacture these chemical weapons makes them the "poor man's weapons of mass destruction". In recent years, these weapons are used frequently because of low cost production and lack of antidote to chemical weapons. During the Iraq–Iran war (1981–1989), vast of chemical weapons as an example mustard gas has caused serious injuries, morbidity, mortality, toxicity and chronic side effects in vital organs such as eye, skin and especially the respiratory system. Mustard gas commonly is a class of relate to cytotoxic and vesicant chemical warfare with the power to form wide blisters on the exposed skin and in the lungs (Nishimoto *et al.*, 1983, 1988; Yamakido *et al.*, 1996). Pure sulfur mustards are colorless, viscous liquids at room temperature. Sulfur Mustard gas (bis, 2-chloroethyl-sulphid) is a strong Alkylation agent like opium/fix. Inhalation of the mustard gas (MG), a bi-functional alkylating agent will cause severe lung damage because of the mutagenic and carcinogenic alkylating agents which can targets the DNA. Mustard gas formula is ($SCl2 + 2\ C_2H_4 \rightarrow (Cl\text{-}CH_2CH_2)_2S$). It can be synthesized by treating sulfur dichloride with ethylene in Depretz (Balali, 2005).

Mustard gas creates a very reactive intermediate (Figure1) and tends to permanently alkylate the guanine nucleotide in DNA strands, which prevents cellular division and causes the initiation of programmed cell death or apoptosis and the

damaged DNA will have caused the development of lung cancer (Khalili, 2009 and Ghanei, 2010).

Figure1.1: Chemical structure. Mustard gas (bis (chloroethyl) thioether) alkylating a DNA amine base. This compound can eliminate a chloride ion via intermolecular nucleophilic replacing to create a cyclic sulfonium ion. Through binding to the amino group of the DNA strand causes damage to DNA (www.bt.cdc.gov/agent/sulfurmustard)

Mustard gas is an alkylating compound that is able to exchange hydrogen for an alkyl group to a specific place on a molecule. Alkylating agents assault the nitrogen, oxygen or sulfur molecule. Common alkylating agents are: Methyl iodide (or other haloalkanes), dimethylsulfate and some carboxylic acid alkyl esters. These chemical compounds link with various nucleophilic groups in nucleic acids and proteins, causing mutagenic, carcinogenic, or cytotoxic effects associated cancer (Bignold, 2006). There is evidence that the carcinogenicity of SM gas activates by genotoxic mechanism of action that complicates DNA alkylation leading to cross-link configuration and inhibition of DNA synthesis and repair, point mutation. Mustard gas makes cancer of the lung (Figure 1.2).

The protein 53 or tumor protein 53 (known as human p53) is a tumor suppressor protein that is encoded by the TP53 gene and is involved in the pathogenesis of malignant disease. The genetic abnormalities of the TP53 gene (p53) are the most common cause of lung cancer. This is because of the P53 mechanism including anticancer function, and has a role in apoptosis, genomic firmness, and inhibition of angiogenesis (http://www.p53.free.fr) TP53 is used as a marker for early detection, prognosis and as a therapeutic option.

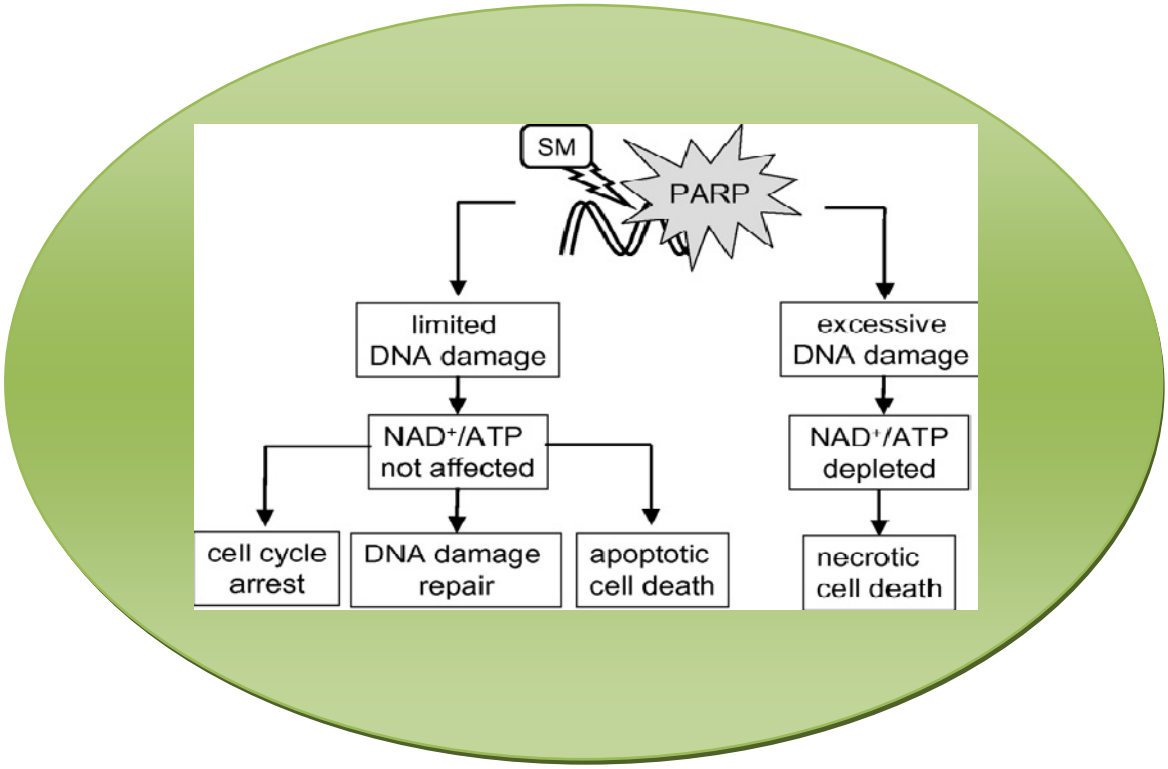

Figure 1.2: The sulfur mustard induces DNA damage. A high concentration of sulfur mustard (SM) over activates PARP (poly-ADP ribolysation of cellular proteins). with diminish NAD+ and ATP, it eventuate to necrotic cell death.DNA damage due to SM does via activation of PARP, but does not the intracellular NAD+ pool. Activated PARP undergoes auto-modification, so it can cause cell cycle arrest, DNA damage repair and apoptotic cell death (Kehek *et al*., 2009).

Fundamentally, inactivation of the p53 gene is pro or due to small mutations (missense and nonsense mutations or insertions/deletions of several nucleotides). The most common mutations critically damage sequence-specific DNA-binding and transactivation, especially the TP53 missense mutations in human cancers appertains with their functional effects (Joerger *et al.*, 2006). In humans, TP53 gene located on the short arm of chromosome 17 (17p13.1) and has 393 amino acids. Alkylating agents has been shown to activate the mutant p53 that are used for cancer therapy. Studies have shown that the MG affects the p53 by causing mutations within exon 5-8. These mutations were predominately G to A transition. In some cases, there were multiple p53 point mutations (Khalili, 2009). Other research has shown the presence of mutations in exon 4-9 or an alteration at codon 278 (CCT→CCA) (Karami, 2007).

1.2 Problem statement

Currently, there is limited knowledge on how the alkylating agent of the mustard gas affects the five domains of p53 3D structures and it potential role as a gene suppressor affecting the process of apoptosis. Moreover to date, there is no any bioinformatics analysis of damaged 3D structure of p53 by alkylation agents as which can demonstrate a precise modeling and simulating of them upon to mutations. In addition, majority of collected data which came from clinical and laboratory experiments depicted p53 manipulated by alkylation agents at transcription level with few indication of these results on post-translation and modification of protein structure. In addition, the types of mutation that can occur on the p53 domains caused by the alkylation factors are not well understood. Thus, building a precise model of these manipulations can be useful for better understanding of p53 mutated structure and designing new effective drugs where final structure of protein is targeted.

1.3 Aim of the study

The purpose of this study is to investigate the possible effects of sulfur mustard on the tertiary structure of proteins 53 as well as study the domains of the Tp53 that are more susceptible to conformation changes due to alkylation effects. The data for this project will be sourced by both bioinformatics databases and experimental results available in the public domain. The data that is gathered from these databases will be analyzed using bioinformatics software to simulate the effect of alkylation agent.

1.4 Objectives of the study

1.3.1 Finding out the effects of SM gas on Tp53 and protein structure and dynamics

1.3.2 Determining the sequence, domain and conformation of the mutated of p53 and modeling the type of mutated

1.3.3 Comparing and analyzing the structural dynamics between the Wild type and mutation of the p53 due to the effect of MG

1.5 Significance of the study

This study will help to understand the MG effects on the p53 structure and conformational changes via simulation. The obtained output enables to interpret useful information regarding the alkylating agent pathway on DNA. On the other hand, data will help us about reverse mutation via simulating and modeling of the mutation by using bioinformatics' tools.

1.6 Scope of the study

In this research, the focus will be on Tp53 mutation in lung cancer that related to effects of MG. The information will be gathered from both bioinformatics databases and experimental results. The data will be mined, mapped and modeled using bioinformatics web-based tools and at the end, comparing the protein structure of the mutated and wild type of Tp53.

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