Deanship of Graduate Studies

Al-Quds University

Expression of Activation Induced Cytidine Deaminase (AID) In Lung Cancer

Kawthar Ali Saleh Qadamani

M.Sc. Thesis

Jerusalem - Palestine

1434 Hijri\ 2013 AD

Expression of Activation Induced Cytidine Deaminase (AID) In Lung Cancer

Prepared By:

Kawthar Ali Saleh Qadamani

***B*.Sc. Biology &Medical Technology**

Al-Quds University - Jerusalem

Supervisor: Dr. Rula Abdul-Ghani

A Thesis Submitted in Partial Fulfillment of the Requirements For the Degree of Master of Medical Sciences in Biochemistry and Molecular Biology. From the Faculty of Graduate Studies, at Al-Quds University, Jerusalem, Palestine.

1434 Hijri\ 2013 AD

Al-Quds University

Deanship of Graduate Studies

Department of Biochemistry and Molecular Biology

Thesis Approval

Expression of Activation Induced Cytidine Deaminase (AID) In Lung Cancer

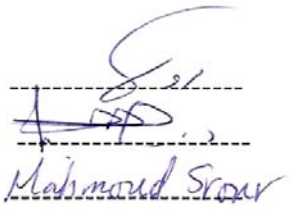
Prepared by: Kawthar Ali Saleh Qadamani

Registration Number: 21012535

Master thesis submitted and accepted, Date: Saturday 8\ 6\2013

The names and signatures of the examining committee members are as follows:

1. Head of committee: Dr. Rula Abdul-Ghani
2. External Examiner: Dr. Riyad Shreim
3. Internal Examiner: Dr. Mahmoud Srour



Jerusalem/Palestine

1434 Hijri/2013 AD

Dedication

I dedicate this thesis to:

To my Dad Ali Qadamani, and my Mom.

To my Husband Abdelraheem Abulafi.

To my father in law Ahmed Abulafi, and my mother in law.

To my wonderfull kids Yasmeen and Ahmed.

And to the rest of the family.

Without their inspiration, motivation, and unconditioned love, none of this would be happened.

Declaration

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Name: Kawthar Ali Saleh Qadamani.

Signature: ----------

Date: 8\6\2013

Acknowledgment

I would like to express my sincere thanks and gratitude to my perfect supervisor Dr. Rula Abdul-Ghani for her intensive support and assistance.

My thanks also go for the pathology department at Al-Maqassed hospital for providing us with Lung Cancer samples.

And thanks for Dr. Riad Shreim and Dr. Marwan Qabajah, Fadwa Zawahreh, and Einas, at the pathology department at Al-Hussein hospital in Beit Jala for their cooperation and assistant.

Thanks for Mrs. Shafeqa Qawasmeh at Hadassah Hospital for training me the immunohistochemistry technique. And thanks for Jameleh Qadi and Dina Ali at Al-Quds University for helping in using microtome and learning some technical aspects.

I want also to thank Hanan Jaffal, Huda Jaffal, Lamya Halasseh, and Dr. Omar Taha at Al-Quds University for their pleasant partnership in the Lab…

Abstract

Lung cancer (LC) is the most common cancer worldwide, affecting over a million people every year. It is the leading cause of cancer-related death in the world, and the diagnosis of this disease is usually associated with poor prognosis. In Palestine, according to the Palestinian Cancer Registry (PCR), lung cancer has been considered the fourth common cancer morbidity in the general population, and considered the first common cancer mortality in the general population.

Activation induced cytidine deaminase (AID), a member of the cytidine deaminase family, is expressed in activated B cells and is involved in antibody diversification by inducing the mutations of immunoglobulin genes. The role of AID in immune response is essential for somatic hypermutation & class switch recombination in B lymphocytes. Although the primary and physiologically relevant targets for AID are immunoglobulins, AID has been shown to attack non-immunoglobulin genes and induce mutations broadly throughout the genome. This property of AID; acting as a DNA mutator, is considered a double edge sword in cellular metabolism. Several studies have shown that AID expression is associated with non-Hodgkin lymphoma and follicular lymphoma as well as non lymphoid cancers such as gastric cancer, hepatocellular carcinoma, and ulcerative colitis-associated carcinoma.

The aim of this study is to investigate the expression levels of AID protein in several types of LC in Palestinian patients, to check if AID is aberrantly expressed in these types. To achieve this aim, parrafin blocks of 63 LC cases with their clinico-pathological data, were collected retrospectively (from 2000-2011) from patients' medical files from the pathology department of Al-Maqassed hospital. And the expression levels of AID were examined in all these cases by immunohistochemistry (IHC) analysis.

Aberrant AID protein expression was detected in 22.2% (13 of 58) of the LC cases. The results showed that AID protein was expressed in 18% (10 of 50) of non-small cell lung carcinoma (NSCLC) and 100% (3 of 3) of small cell lung carcinoma (SCLC). Moreover 23% (7 of 31) of squamous cell carcinoma and 13% (3 of 24) of adenocarcinoma express AID protein. No correlation was detected between AID protein expression and clinic-pathological data of the patients such as gender, age, LC type, tumor grade. In conclusion, examination of AID protein expression in LC revealed aberrant expression of AID protein in a subset of LC Palestinian patients.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Ab	Antibody
Ag	Antigen
AID	Activation Induced Cytidine deaminase
APOBEC	Apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like.
CSR	Class Switch Recombination
DAB	3'3' diaminobenzedine
DNA	Deoxyribonucleic Acid
F	Female
H&E	Hematoxylin and Eosin
H. Pylori	*Helicobacter Pylori*
HCC	Hepatocellular Carcinoma
Ig	Immunoglobulin
IHC	Immunohistochemistry
IL	Interleukin
LC	Lung Cancer
M	Male
NF-kb	Nuclear Factor Kappa-light-chain-enhancer of Activated B cells
NLS	Nuclear Localization Signal
NS	Non-Smoker
NSCLC	Non-Small Cell Lung Cancer
RNA	Ribonucleic Acid
S	Smoker
SCLC	Small Cell Lung Cancer
SHM	Somatic Hypermutation
TBS	Tris Buffer Saline
TNF-α	Tumor Necrosis Factor

Chapter One: Introduction

1.1. Lung Cancer:

Lung Cancer (LC) like other types of cancer is a multistage process involving alterations in multiple genes and diverse pathways (Devereux et al., 1996). These mutations can cause activation of cancer promoting genes (oncogenes) or switching off cancer protecting genes (tumor suppressors). Several mutations in different genes may be required to turn a normal cell into a cancerous one. At first the changes to a cell may be reversible but as more mutations accumulate, the changes become irreversible and a cancerous cell develops (Collins et al., 2007; Gilligan and Rintoul, 2007). The two major types of LC are non-small cell lung cancer NSCLC (about 80% of LC) and small cell lung cancers SCLC (about 20%). The tumor starts off in the lungs and although, initially, it may be limited to one lung, it will eventually spread to other parts of the body (Gilligan and Rintoul, 2007).

1.1.1. Epidemiology of Lung Cancer:

According to the Palestinian Cancer Registry (PCR), the most common cancer morbidity in the general population was breast (15.7%), lymphomas (9.1%), bone marrow (9.1%), bronchus and lung (8.7%), colorectal (7.4%), brain and other nervous system (4.8%), urinary bladder (4.7%), stomach (3.5%), liver (3.3%), and prostate (2.9%). In males, bronchus and lung cancer was the first leading cause of cancer morbidity (14.7%). In females, breast cancer (30%) was the first cause of morbidity (Abdeen, 2006).

As for mortality rates, the most common cancer mortality in the general population was bronchus and lung (12.7%), breast (11.1%), bone marrow (9.9%), lymphomas (7.2%), colorectal (7.1%), urinary bladder (3.9%), liver (5.2%), stomach (4.2%), brain and other nervous system (5.8%) and pancreas (3.8%). In males, bronchus and lung cancer was the first leading cause of cancer mortality (19.9%), and breast cancer (23.1%) was the first

cause of mortality in females. Median age at death for all cancer types was 59 years (63 years for male and 54 year for female) (Abdeen, 2006; MOH, 2002).

In Gaza, the incidence rate of breast cancer is 60 per 100,000 population making it the most prevalent type of cancer (16.4%) in the total population according to Cancer Registry Center. Lung cancer was the most prevalent type of males' cancer (12.7%), while breast cancer occupied the most prevalent type in females (31%) (Abdeen, 2006; MOH, 2002).

1.1.2. Risk Factors of Lung Cancer:

LC represents the prototype of a cancer induced by chemical and environmental carcinogens. Smoking is the major risk factor of LC, accounting for about 90% of LC incidence (Biesalski et al., 1998). Several exogenous and endogenous risk factors have been shown to contribute to LC development. Although LC can occur at any age, the risk of developing LC increases with age. Lung cancer is uncommon under the age of 50 years and three quarters of causes occur over the age of 63 years old (Gilligan and Rintoul , 2007).

Smoking causes the vast majority of the cases of LC. About nine out of ten people who develop LC are, or have been smokers. Passive smoking (environmental tobacco smoke) is also considered as a human carcinogen. A non-smoker who is married to a smoker has up to a 30% greater risk of developing LC compared to someone who is married to a non-smoker. Exposure to non-tobacco carcinogens such as radon, asbestos, and chemical carcinogens such as diesel fumes, uranium, nickel, arsenic, zinc, polycyclic hydrocarbons, and chromium, stimulate LC (Gilligan and Rintoul, 2007; Biesalski et al., 1998).

There is some evidence that people who has had previous radiotherapy to their chest or for Hodgkin's disease may be at increased risk of LC, especially if they smoke, or have other risk factors. People who have scarring within their lungs from other causes, e.g. Lung fibrosis, seem to be particularly prone to developing LC especially if they also smoke (Gilligan and Rintoul, 2007). Brothers, sisters and children of those who have had LC may be at a slightly higher risk of developing LC themselves (Collins et al., 2007).

1.1.3. Types of Lung Cancer:

Around 95% of primary LC is epithelial in origin, and these are categorized on the basis of histological appearance into; NSCLC and SCLC (Hirsch et al., 2001). NSCLC accounts for about 80% of cases of LC, and SCLC for the remaining 20%. Both types usually behave differently and respond to treatment differently (Herbst et al., 2008; Gilligan and Rintoul, 2007).

NSCLC:

NSCLC is usually divided into three different types depending on its appearance under the microscope squamous cell carcinoma, adenocarcinoma, large cell carcinoma. Squamous cell carcinoma is the most common type of NSCLC. This type tends to grow more slowly than the other types of LC and spreads at a later stage. Adenocarcinoma can spread to the lymph glands or to other parts of the body at an early stage, it is the most common type to occur in people who have never smoked. Large cell carcinoma accounts for about 10% of all lung cancers. and found in smokers, it has high tendency to spread to lymph nodes (Gilligan and Rintoul, 2007; Travis, 2002; Herbst et al., 2008).

SCLC:

SCLC as the name implies, the cells of this type appear small under the microscope and are largely filled by the nucleus. Small cell carcinoma can occur in any organ but the great majority develops in the lung (Terada, 2012). Almost all SCLC cases are caused by smoking; it is rare for someone who has never smoked to develop it. It is an aggressive type of LC and in the majority of cases it spreads to the lymph glands or to other parts of the body by the time it is discovered (Gilligan and Rintoul, 2007).

Other types of LC include hamartoma and carcinoid tumors. Hamartomas are the most common type of benign (non-cancerous) lung tumor. They are usually quite small, and mostly cause no symptoms. They never become cancerous or reoccur following removal (Gilligan and Rintoul, 2007).

Carcinoid tumors are a rare form of tumor that can occur in various parts of the body including the lung. They belong to the family of neuroendocrine tumors (Gilligan and Rintoul, 2007). The symptoms that they cause tend to depend on their location within the lung (Gilligan and Rintoul, 2007).

1.1.4. Symptoms of LC:

The most common symptoms that can occur in the late stage LC include; persistent cough, shortness of breath, coughing up blood (haemoptysis), repeated chest infections that are slow to clear up, tiredness and fatigue, noisy breathing, horse voice, unexplained weight loss, and new aches or pains in the chest or shoulders (Gilligan and Rintoul, 2007).

1.1.5. Staging of Lung Cancer:

The accurate staging of LC provides both prognostic information and appropriate treatment to the patients. Once a LC has been diagnosed, it is important to know exactly which part of the lung it is laying in, how big it is, and whether it has spread in order to know the cancer's stage and the appropriate treatment. The staging systems used for NSCLC and SCLC are different. The international classification system for NSCLC types is called the TNM system, this stands for tumor (T), lymph nodes (N) and metastasis (M). SCLC is divided into two stages, limited stage where the tumor is limited to one lung and lymph glands in the centre of the chest or the root of the neck, and extensive stage where the tumor has spread beyond the lung it started in to more distant lymph glands or other organs in the body such as the liver or the brain (Gilligan and Rintoul, 2007; Mountain, 2008).

1.1.6. Diagnosis and Treatment of LC:

Chest radiograph is the first diagnostic step for patients that have reported suspected symptoms of LC. This test may show some obvious mass, widening of the mediastinum, atelectasis, consolidation, or pleural effusion. CT imaging can provide more information about the type and extent of disease. Bronchoscopy or CT-guided biopsy is often used to sample the tumor for histopathology (Collins et al., 2007).

LC is treated by surgery and adjuvant chemotherapy for early stage disease, concurrent chemoradiotherapy for locally advanced disease, and chemotherapy for metastatic disease (Pandya et al., 2007; Salgia and Skarin, 2012). Nevertheless treatment differs according to the histologic type of cancer, the stage at presentation, and the patient's functional evaluation (Collins, 2007; Salgia and Skarin, 2012).

1.1.7. Genetic Alterations in Lung Cancer:

Lung tumorigenesis is a complex multi process that involves frequent genetic alterations that activate oncogenes and inactivate tumor suppressor genes (Devereux et al., 1996). Most, if not all, of these alterations are considered as being critical for changing the phenotype of non-cancerous cells or cancer cells to more malignant ones by stepwise progression. Thus, genetic alterations responsible for each step of phenotypic change in cancer cells can be considered as molecular footprints as long as the cells retain malignant phenotypes (Yokota et al., 2004).

Genes involved in DNA repair, cell growth, signal transduction, and cell cycle control may all be damaged at different stages of lung tumor progression. Mutational activation of oncogenes, inactivation of tumor suppressor genes, and subsequent increased genetic instability are major genetic events in lung carcinogenesis (Yokota et al., 2004).

1.1.7.1. Chromosomal Abnormalities:

Non-random chromosomal abnormalities have been identified in LC cell lines and fresh tissues, including loss of chromosomes 3p, 9p, and 17p. Three distinct 3p regions may be affected, including 3p25, 3p21.3, and 3p14-cent. The loss of alleles at 3p is observed in greater than 90% of SCLC tumors and approximately 50% of NSCLC tumors (Salgia R et al., 2012). Initially, putative candidates on 3p included the c-RAF-1 proto-oncogene, the B-retnoic acid receptor gene, zinc finger containing genes, the protein tyrosine phosphatase-y gene, and the von Hippel Lindau tumor suppressor gene at 3p14-23. Most recently studied, the Fragile Histidine Triad (FHIT) gene localized to 3p14.2, and in one study approximately 80% of SCLC tumors showed abnormalities of this gene. Loss of the FHIT gene leads to the stimulation of DNA synthesis and proliferation (Sozzi et al., 1996).

In NSCLC other genetic losses include genetic loss at chromosome 8p, which may occur in 50% of tumor samples. Genetic loss at 9p involves the p16 and p15 tumor suppressor genes may occur in 67% of tumor samples. Genetic loss at 11p (p13 and p15) sometimes results in deletion of the Wilms' tumor suppressor gene at region p13 and may occur in 20% to 46% of tumor samples (Salgia et al., 2012; Pandya et al., 2007).

Telomerase activity has been directly correlated with malignant and metastatic phenotype of a wide array of solid tumors, and many studies on LC tissues showed that most of the cases had high telomerase activity (Salgia R et al., 2012; Devereux TR et al., 1996).

1.1.7.2. Expression of Oncogenes:

The most common oncogens that have been associated with LC include RAS, MYC, c-erbB-2, and BCL-2.

RAS. The rat sarcoma viral oncogene homolog (RAS) family of proto-oncogenes (KRAS, NRAS, and HRAS) encodes membrane-bound GTPases that play an important role in signal transduction and cellular proliferation. Ras is activated by point mutations in up to 35% of LC, and is associated with smoking. Oncogenic mutations of KRas, commonly located in codons 12, 13, and 61, lead to increased GTP binding and constitutive activation of KRas and the MAP kinase pathway. Oncogeneic KRas is sufficient to expand murine bronchioalveolar stem cells in culture and in vivo (Pandya et al., 2007).

MYC. The MYC dominant oncogenes, C-MYC (cellular), N-MYC (originally isolated from neuroblastoma cells), and L-MYC (originally isolated from SCLC cells), encode for nuclear DNA-binding proteins, which are involved in transcriptional regulation (Salgia et al., 2012). Amplification of MYC family proto-oncogenes is found in almost all small-cell lung cancers (SCLC) and is reported to confer an aggressive, resistant phenotype (Pandya et al., 2007).

c-erbB-2. c-erbB-1 proto-oncogen encodes the epidermal growth factor receptor (EGFR) and has been a classic model for signal transduction events in normal and transformed cells. A related proto-oncogene, c-erbB-2 (also known as HER-2\neu) encodes for a protein product that is also a growth factor receptor (Salgia et al., 2012). Strong EGFR expression is present in 40% to 80% of NSCLC tumor specimens. High-level expression of EGFR is also present in premalignant bronchial epithelium, suggesting a role for EGFR in carcinogenesis (Pandya et al., 2007).

BCL-2. BCL-2 is a negative regulator of cell death, prolongs the survival of noncycling cells, and inhibits apoptosis of cycling cells (Salgia et al., 2012).

1.1.7.3. Loss of Tumor Suppressor Genes:

The most common tumor suppressor genes that have been associated with LC include RB, p53, and p16INK4A.

RB. The RB gene encodes for a nuclear protein that was initially determined to be abnormal in patients with retinoblastoma. SCLC besides retinoblastoma was a tumor initially identified to have abnormalities of RB. In SCLC cell lines, there is rearrangement of chromosome 13, directly affecting the 13q14 locus where RB is located. Up to 70% of SCLC cell lines have alterations in RB gene or abnormal mRNA, which thereby leads to absent expression of the protein (Salgia et al., 2012).

p53. The p53 tumor suppressor gene is the most commonly mutated gene in human cancer. The highest incidence of p53 mutations in lung cancer is in SCLC. Loss of p53 function or one of the genes in the p53 pathway of cellular growth control, can lead to unchecked progression of the cell cycle before appropriate DNA repair can occur (Salgia et al., 2012; Devereux et al., 1996). Although p53 alterations occur in the late stages of some cancers, they appear early in NSCLC suggesting that this gene may be damaged early in the genesis of LC and may provide a selective advantage for cell growth (Devereux et al., 1996).

P16INK4A. Some LC specimens have deletion of chromosome 9p21, thus implicating one or more tumor suppressor genes in this region as being important, and from genetic analysis, p16INK4A has been identified to map within this region. Moreover, the expression of *P16INK4A* has been evaluated in LC cases (Salgia et al., 2012; Herbst et al., 2008).

1.1.8. Tumor Markers for Lung Cancer:

Molecular markers are important tools for understanding the induction of cancer, early diagnosis, prognosis and identifying new treatment for cancer patients. They are non-invasive, reproducible, and fast diagnostic tools in differential diagnosis and in monitoring therapy efficacy. Further research is necessary to uncover novel biomarkers that can help in the fight against cancer. The screening and investigation of their prognostic value is of great importance (Gilligan and Rintoul, 2007).

The determination of tumor markers at the time of primary diagnosis may be helpful because tumor markers expressed and released at the time of primary diagnosis are likely to be the most relevant markers for follow up monitoring. Moreover the rate and extent of decrease of preoperatively released markers after surgery provide useful information about remaining tumor burden and the effectiveness of therapy (Erbt et al., 1994).

There are general tissue-based and serum-based tumor markers used for LC screening and diagnosis such as:

- *Neuron specific enolase (NSE).* Numerous studies have supported its use in the diagnosis of SCLC. However, it does not have the sensitivity or specificity necessary for use in population screening (Shibayama T et al., 2001).
- *Carcinoembryonic antigen (CEA).* Its levels are high in adenocarcinoma and large cell carcinoma, but the elevated levels are also detectable in various benign pathologies and other malignancies preclude its use in screening and limit its diagnostic use (Ebert W et al., 1994).
- *Cytokeratin-19 fragments (CYFRA 21-1).* It is the most sensitive tumor marker for NSCLC, particularly squamous cell tumors (Ebert W et al., 1994).
- *Progastrin- releasing peptide (ProGRP).* It is a reliable marker for SCLC with good specificity and sensitivity. ProGRP has shown to be helpful in differential diagnosis, particularly in distinguishing SCLC from other LC types (Yamaguchi K et al., 1995, Stieber P et al.; Schalhorn A et al., 2001).
- *Squamous cell carcinoma antigen (SCCA).* This marker has superior specificity for squamous cell cancer and can be used for histological subtyping (Ebert W et al., 1994).

1.2. Activation-induced Cytidine Deaminase (AID):

1.2.1. The AID and APOBEC Family:

APOBEC is the Apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like. It is a family of evolutionary conserved proteins that can insert mutations in DNA and RNA as a result of their ability to deaminate cytidine to uridine (Figure 1-b) (Holmes RK et al., 2007). In humans, the APOBEC family consists of 11 members, including APOBEC1, APOBEC2, APOBEC3 (A-H), APOBEC4 and AID. All the APOBECs share the structural and catalytic backbone of the zinc-dependent deaminases, they have a zinc-coordination motif which forms the core of the catalytic site (Figure 1-a) (Larijani et al., 2012).

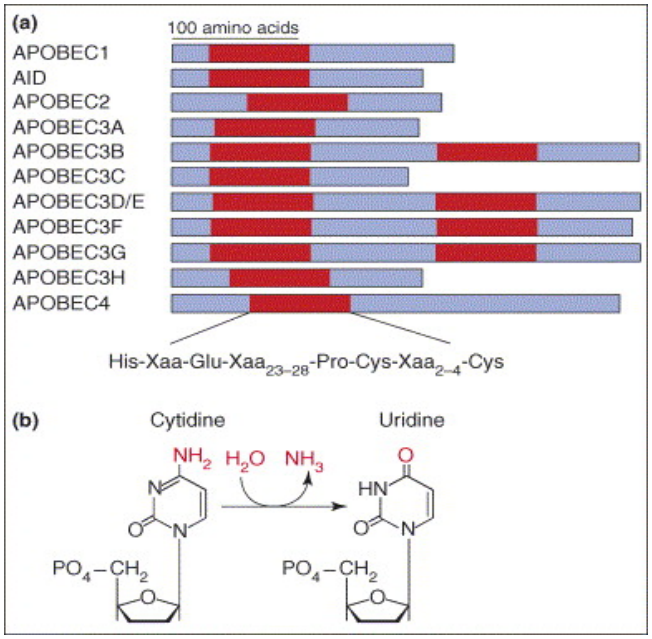


Figure 1: The Human APOBEC Family of Proteins. (a) Domain organization of human APOBEC proteins. Red shading indicates the cytidine deaminase motifs in each protein; the consensus amino acid sequence is shown below. **(b)** The cytidine deamination reaction catalysed by APOBEC enzymes. In this process, cytidine is converted to uridine by the addition of water and the removal of an amine group (Holmes et al., 2007).

Another member of the family, activation induced cytidine deaminase (AID), was discovered in 1999 by Muramatsu et al. AID is encoded by the A*cida* gene on chromosome 12 (Revy et al., 2000). AID has been shown to be selectively expressed in germinal centers

of activated B cells (Revy et al., 2000). It is known to be a key molecule for generating 'antibody memory' (Nagaoka et al., 2010), and essential for the antigen-driven diversification of immunoglobulin genes in the vertebrate adaptive immune system (Conicello, 2008). Genetic AID deficiency leads to Type 2 hyper-IgM syndrome, an immunodeficincey in which the inability to carry out class switch recombination leads to the absence of antibodies other than those of the IgM class (Conicello, 2008). AID deficiency has also been found to result in a range of other abnormalities such as autoimmunity and lymphoid hyperplasia (Meyers et al., 2011, Kuraok et al., 2011).

Recently the tertiary structure of the human AID protein was modeled as shown in figure 2. The model shows that AID protein has a core of 5 beta strands surrounded by 6 alpha helices with the Zn-coordinating residues (Larijani et al., 2012).

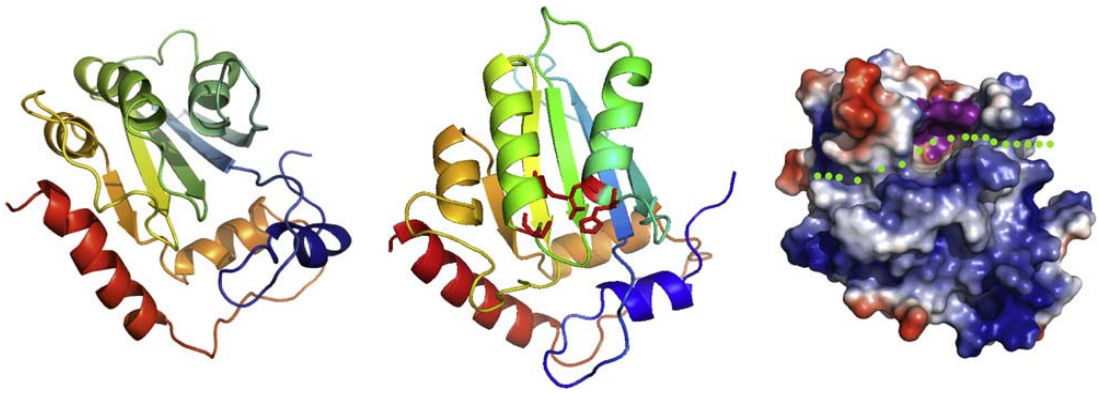


Figure 2: The Three Dimensional Model of AID Protein. The left panel shows a ribbon model of the predicted structures of human AID. N- to C-terminus progression is shown in color from blue to red. The middle panel shows the putative catalytic (Zn- coordinating). The right panel shows a model of the surface topography of AID (Larijani et al., 2012).

1.2.2. Physiological Role of AID:

Efficient humoral immunity to foreign antigens requires that mature B lymphocytes diversify their immunoglobulin genes through class-switch recombination (CSR) and somatic hypermutation (SHM). AID expressed mainly but not exclusively in activated mature B cells has an essential role in the induction of both CSR and SHM. During SHM, AID activity induces a very high rate of mutation of genes encoding the immunoglobulin heavy-chain and light-chain variable regions to generate immunoglobulins with greater antigen affinity (Lee-Theilen and Chaudhuri, 2010). AID has two separate functions in CSR; the DNA cleavage of target loci and the correct pairing of cleaved ends. The DNA

cleavage activity is localized to the N-terminal portion of AID protein, and the C-terminal portion of AID is responsible for appropriately pairing the cleaved DNA ends (Nagaoka et al., 2010). Although a lack of AID activity leads to immunodeficiency syndromes, higher AID expression results in malignant transformation of B cells and T cells as well as tumor development in various organs (lee-Theilen and Chaudhuri, 2010).

1.2.3. Pathological Role of AID in Cancer:

AID was initially thought to be an RNA-editing enzyme, but the discovery that it could mutate E. coli DNA with a preference for targeting C nucleotides, provided insight into its mechanism of action (Honjo et al., 2002). The DNA deamination model proposes that AID binds to target DNA and deaminates dC to dU, thus creating a U-G mismatch. This triggers the base excision repair pathway, consisting of uracil DNA glycosylase (enzyme responsible for the removale of uracil in DNA, and acts downstream of AID) and apurinic-apyrimidic endonuclease, followed by the activation of error-prone DNA repair pathways (Figure 3). On the other hand, the RNA editing hypothesis proposes that AID edits RNA, and the resultant product is involved in DNA cleavage at the target loci (Nagaoka et al., 2010). It is clear that DNA-cleavage activity of AID does not have any specific recognition sequence because neither the V nor S region (regions on the DNA responsible for antibody diversity) contains any consensus cleavage site. In addition, no clear binding specificity of AID to DNA has been demonstrated (Robbiani et al., 2009).

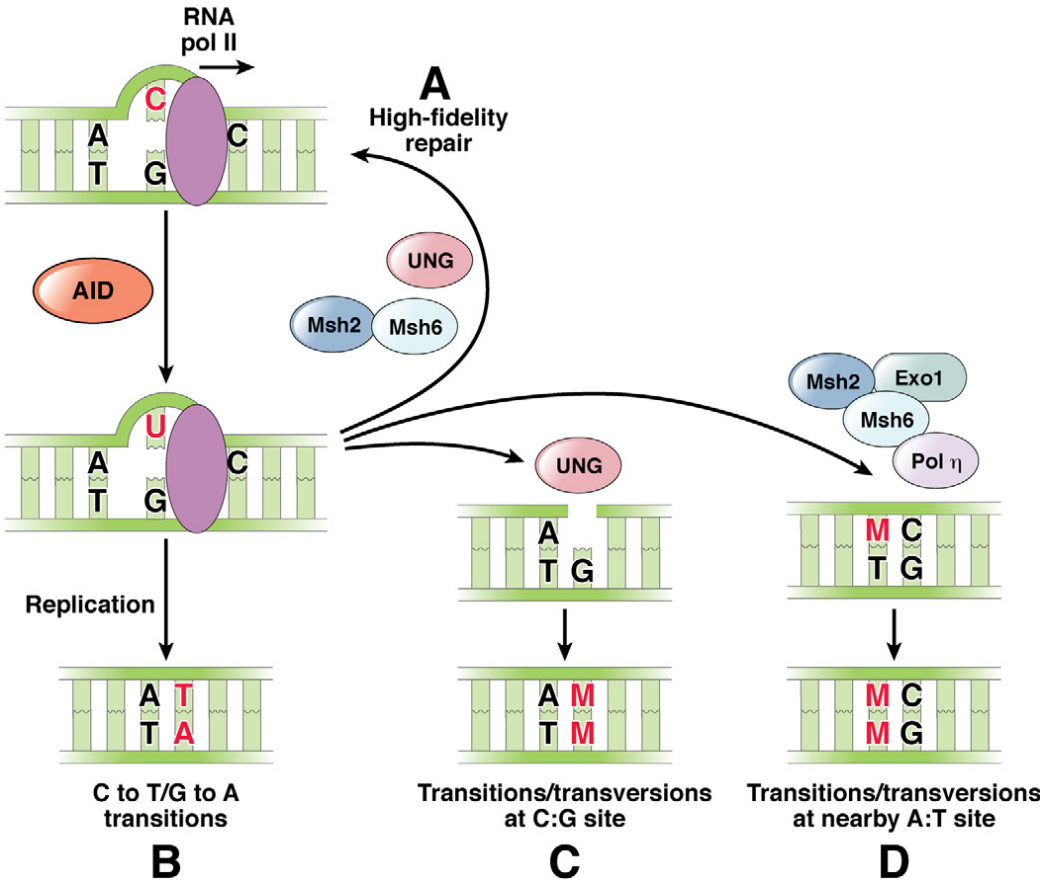


Figure 3: Mechanism of Mutation Induction by AID Activity. AID deaminates cytosine (C), resulting in the generation of a uracil (U), and therefore can transform a DNA C:G pair into a U:G mismatch. **(*A*)** The AID-generated U:G mismatch can be recognized by uracil-DNA-glycosylase (UNG) or MSH2/MSH6 heterodimer and repaired correctly. **(*B*)** If DNA replication starts before recognition by the repair system, a U:G mismatch gives rise to C/G to T/A transition. Alternatively, **(*C*)** generation of an abasic site by UNG or **(*D*)** recognition of the U:G mismatch by the MSH2/MSH6 heterodimer induces any mutations in the AID-generated U:G mismatch or at a nearby A:T site, respectively, in an error-prone manner (indicated as M) (Chiba T et al., 2012).

Fortunately, AID activity is tightly regulated/ restricted to activated-normal B-lymphocytes and no activity is detected in normal tissues under normal physiologic conditions. However the role of AID as a DNA mutator enzyme led to the hypothesis that AID might induce inappropriate mutations in non-Ig genes. Evidence for this hypothesis came from a study on murine cell lines, the study showed that several genes e.g. myc, bc16, pim1, pax5 …etc are deaminated by AID and are repaired by mismatch and base excision repair pathways, but around 25% were not fully protected by the repair mechanisms and accumulated mutations (liu et al., 2008).

The generation of AID transgenic mice provided insight into the role of AID in tumorgenesis. Several reports on these transgenic mice showed that they develop lymphoma, gastric carcinoma, and hepatocellular carcinoma (Okazaki et al., 2003, Matsumoto et al., 2007, Takai et al., 2009). It has been proposed that AID expression persists in malignant B cells as well as in epithelial tumors and induce genomic instability and tumorgenesis by inserting mutation in tumor-related genes in both lymphoid and non lymphoid organs (Okazaki et al., 2003, Matsumoto et al., 2007, Takai et al., 2009). One of these genes which received great interest is p53, known as the "guardian of the genome". A report by Takai et al., showed that AID transgenic mice which developed hepatocellular carcinoma (HCC) had deleterious p53 mutations (Takai et al., 2009). AID-induced p53 mutations were also reported in gastric epithelial cells (Matsumoto Y et al., 2007). Furthermore, somatic hypermutations in p53 gene were identified in B-cell chronic leukemia (Malcikova et al., 2008). These studies led to the conclusion that AID is an inducer of p53 mutagenesis in cancer cells (Takaishi et al., 2007).

1.2.4. Regulation of AID Expression:

As a potent mutator, AID is under stringent transcriptional, post-transcriptional, and post-translational regulation. Transcription factors such as HoxC4 an NF-κB which are upregulated in a B-cells upregulate the induction of AID (Xu et al., 2007). These transcription factors are triggered by several factors, some of which are pathogenic e.g. bacteria and viruses (Matsumoto et al., 2007; Endo et al., 2007). A report by Matsumoto et al., 2007 showed that *Helicobacter pylori (H. pylori)* infection caused AID-mediated p53 mutations through an NF-κB activation pathway in gastric epithelial tissue. *H. pylori* introduces several bacterial virulence factors into gastric epithelial cells through a type-IV secretion apparatus, and this have been shown to be responsible for activating the transcription factor NF-κB and then induction of AID expression as shown in figure 4 (Marusawa and Chiba , 2010). Other studies showed that AID is expressed in liver cancers and chronic hepatitis tissue. The introduction of the gene for the core protein of HCV into human hepatocyte induced AID expression via NF-κB activation. This is consistent with

previous studies showing that the core protein of HCV enhances NF-ƙB activation (Marusaua et al., 1999; Kou et al., 2006; Endo et al., 2007).

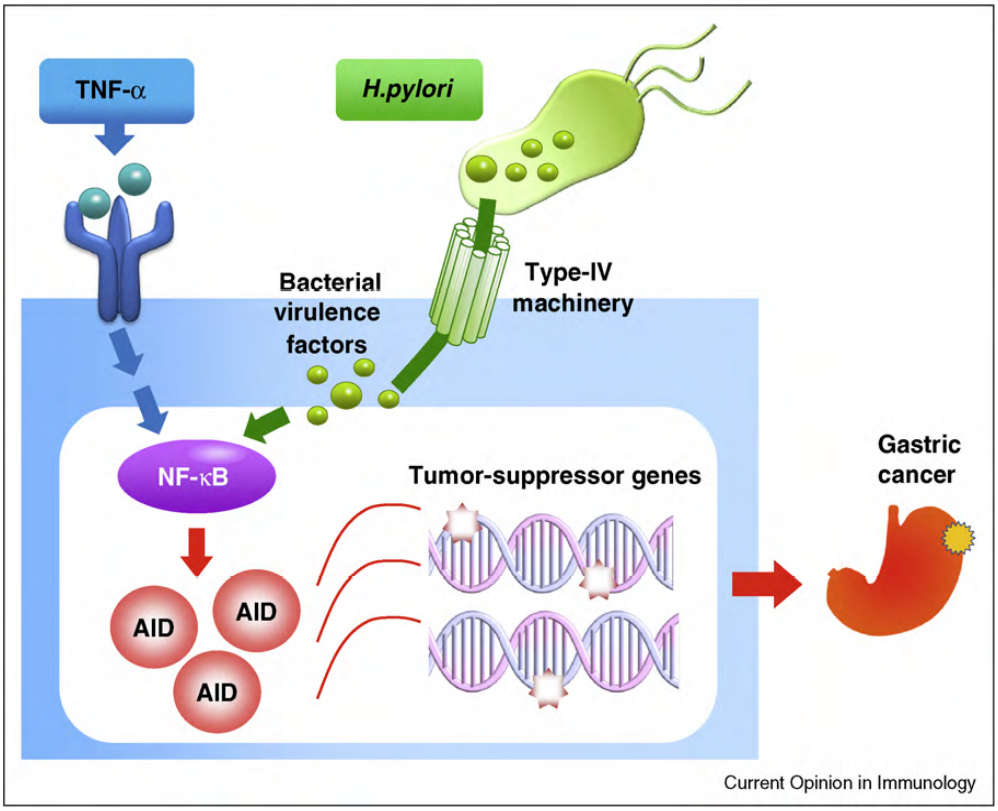


Figure 4: H. pylori Infection Triggers AID Expression In Gastric Epithelial Cells Via Two Distinct Pathways (Marusawa and Chiba, 2010).

It is well known that proinflammatory cytokine induction of AID expression via the NF-kB pathway is not limited to gastric epithelial cells. AID expression is mediated by the inflammatory response in a variety of epithelial cells, including human hepatocytes, and biliary and colonic epithelial cells. Aberrant AID expression in these gastrointestinal organs results in somatic mutations in various tumor-related genes. Thus, AID may have a central role in genetic susceptibility to mutagenesis, which leads to cancers in these gastrointestinal tissues upon exposure to certain inflammation or infection as shown in figure 5 (Marusawa and Chiba, 2010).

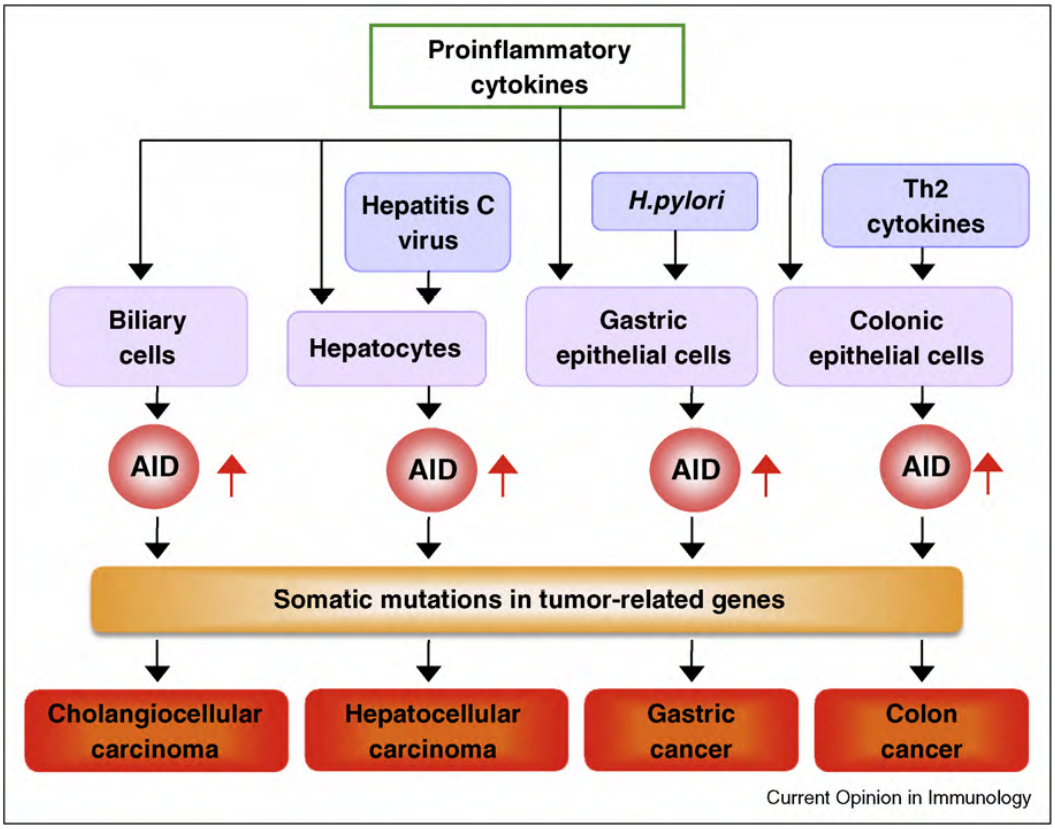


Figure 5: AID Links Inflammation and Infection to Cancer Development in Various Gastrointestinal Tissues (Marusawa and Chiba, 2010).

AID promoter has also been shown to be triggered by the sex hormone estrogen; another transcriptional regulator. Estrogen-estrogen receptor complex was shown to bind to AID promoter enhancing AID's mRNA and protein levels an effect which was inhibited by tamoxifen; an estrogen antagonist. This study suggested clearly that estrogen- induced oncogenesis is derived through AID dependent mutagenesis (Paulkin et al., 2009). These findings might explain why women are more susceptible to autoimmune diseases and cancer particularly in breast tissue (Maul et al., 2009).

AID undergoes a series of post-translational modification, such as nuclear/cytoplasmic translocation, dimerization/oligomerization, and phosphorylation, all of which play important roles in regulating AID activity and function in SHM and CSR (Xu et al., 2007). Many studies suggest that the subcellular localization of AID is tightly controlled to limit the amount of the enzyme in the nucleus (Delker et al., 2009), whereas the function of AID is exerted there, AID is predominantly cytoplasmic owing to the presence of a nuclear export signal (NES) at the extreme carboxyl termins (Conicello, 2008).

1.3. Objectives:

Several studies showed aberrant AID protein expression in several types of cancer. In Palestine, link between cancer and AID, or other oncogenes or tumor suppressor genes is not studied yet. The aim of this study is to check if AID protein is expressed in LC Palestinian patients, and if AID protein level correlates with clinic-pathological data of the patient.

Chapter Two: Materials and Methods

2.1. Materials:

Reagents and buffers used in IHC and H&E staining are listed in table 1.

Table 1: Materials for IHC and H&E Staining.

Material	Company	Catalog #
Xylene	Loba Chemie	Cas #: 1330-20-7
Ethanol	Carlo Erba	64-17-5
Citrate buffer pH=6	Invitrogen	Ref#: 005000
Hydrogen peroxide 3%	MP Biomedicals, USA	Article #: 194057
Pap-pen	Invitrogen	00-8899
TBS buffer	Sigma	T5912-1L
Primary AID Ab	e-Bioscience	14-5959
Cass block	Invetrogen	00-8120
Secondary anti-Rat Ab	Histofine	414311F
DAB chromagen	Cell -Marque	957D-31
DAB buffer substrate	Cell- Marque	957D-32
Hematoxylene	Sigma	GHS3128-4L
Mounting solution	Fluka	03989-10ML
Super Frosted Plus slides	Fisher brand	12-550-15
Eosin Y	Sigma	E6003-25G

2.2. Methods:

2.2.1. Sample Collection:

Archived paraffin embedded blocks of 63 LC cases were collected retrospectively for the last 11 years (from 2000-2011), from the Pathology Department at Al Maqassed Islamic Charitable Hospital in East Jerusalem. Cases were accompanied by their pathological reports obtained from the hospital's records archive. The data include age, gender, year of specimen collection, smoking status, tumor grade, and stage of tumor invasion. The study design proposal was approved by both the Faculty of Medicine, at Al-Quds University, and Al-Maqassed Islamic Charbitale Hospital.

2.2.2. Tissue Sectioning:

LC tissues taken from biopsy must be fixed using formalin, 10% neutral buffered formalin is most commonly used, and fixation between 18-24 hours, this was done be the technician in the Hospital's lab. Under fixation can lead to edge staining, with strong signal on the edges of the section and no signal on the middle, while over fixation can mask the epitope. Antigen retrieval can help overcome this masking, but if the tissue has been fixed for a long period of time, there may be no signal even after antigen retrieval. In this project, 2 slides showed staining at the edges. Some negative cases may be occurred due to over fixation. After fixation tissue blocks must be embedded in paraffin wax and archived for further use.

Tissue sections (5 μm thick slices), from archived paraffin embedded blocks, was cut using the microtome since it is the ideal thickness for IHC, and was mounted on super frosted plus glass slides. These slides electrostatically attract fresh frozen or formalin fixed paraffin embedded tissue sections. One section from each case block was prepared for H&E staining for histological diagnosis and evaluation, and one section for IHC staining.

2.2.3. Standard Staining (H&E):

For each case, standard H&E staining was performed for histological diagnosis and evaluation as shown in table 2 (Avwioro, 2011):

Table 2: H&E Staining Protocol.

Treatment	Time
Xylene 1	2 min
Xylene 2	2 min
Ethanol 100%	1min
Ethanol 80%	1x5sec
Ethanol 70%	1x5sec
Tap water	3 min
Hematoxylin	2-4 min
Tap water	Wash
Eosin	5-6 min
Tap water	3 min
Ethanol 70%	1x5sec
Ethanol 80%	1x5sec
Ethanol 100%	1 min
Mounting solution and cover glass	

2.2.4. Immunohistochemistry (IHC):

IHC has become a valuable tool in both diagnosis and research of infectious and neoplastic diseases in a variety of animals. The basis of IHC is very simple and bridges three scientific disciplines: immunology, histology and chemistry. The fundamental concept behind IHC is the demonstration of antigens (Ag) within tissue sections by means of specific antibodies (Ab). Once antigen-antibody (Ag-Ab) binding occurs, it is demonstrated with a colored histochemical reaction visible by light microscopy or flourochromes with ultraviolet light, and showing the localization of the antigen in the cell (Ramos-Vara, 2005).

For cancer diagnosis, IHC represents an important complementary tool for the routine diagnosis of cancers including LC, and for the identification of the different histological types and prognostic factors. Its purpose is to categorize patients in order to ensure appropriate and specific treatment, as well as to identify tumors at higher risk of recurrence and fatal outcomes. It is better to perform RT-PCR with western blot and IHC for further confirmation, but this needs fresh tissue to be obtained from each case. IHC was performed

because of the availability of archived embedded blocks, and have an advantage of over Western Blott in that it shows the localization of the protein inside the cell, within the tissue.

▪ All tissue sections mounted on superfrosted slides were treated according to the protocol of immunohistochemistry presented in table 3 (Ramos-Vara, 2005):

Table 3: Immunohistochemistry Protocol.

Treatment	Time	Notes
Xylene	3x3min	Deparrafinization (in laminar flow hood)
Ethanol	3x1min	To remove dissolved paraffin wax and Xylene
Ethanol 96%	3x1min	Hydration
Ethanol 80%	3min	Hydration
DDW	3x5sec	Wash
Citrate buffer, pH6	15min, high power, in microwave	To enhance antigen exposure
DDW	3x2min	Wash
3% hydrogen peroxide	15 min	Peroxidase quenching
DDW	2x2min	Wash
Surround/circle the tissue		Use Pap pen
TBS buffer	3x2 min	
Primary AID Ab	Over night	Incubate at 4°C, diluted 1:50
TBS buffer	3x5 min	Wash
Secondary Anti Rat-Ab	30 min	2 drops
TBS buffer	3x5min	Wash
DAB chromagen	25 min	"substrate chromagen"
DDW	5sec	Wash
Hematoxylene	45 sec	Counter stain
Tap water	5sec	Wash
Ethanol 100%	2min	Dehydrate
Xylene 1	2 min	
Xylene 2	2 min	
Mounting solution and cover glass		

▪ Control slides are important for accurate interpretation of results. There are two types of controls, positive control and negative control. Positive tissue control is a specimen, containing the antigen needed, processed at the same time with other slides, to insure that the conditions of the technique were performed accurately. Positive control slides were also used to detect the exact dilution needed for the primary antibody (i.e. for calibration) (Moldenhauer et al., 2006). For AID antibody the positive control used was sections from tonsil tissue kindly provided from Beit Jala Hospital. Tonsils as well as lymph nodes express high levels of cytoplasmic AID protein in B lymphocytes

Negative control/ Reagent control is an additional slide, treated with a buffer instead of one step in the reaction, and processed at the same time with other tissues (Ramos-Vara, 2005). In this study, negative controls were treated with buffer instead of DAB chromagen.

▪ Before proceeding with the staining protocol, the slides must be deparaffinized and rehydrated; incomplete removal of paraffin can cause poor staining of the sections. Therfore, xylene and graduated concentrations of ethanol were used.

▪ When using a new antibody in IHC protocol, the antibody must be calibrated to find the optimal staining conditions. Each antigen has a preferred method of antigen retrieval, and each antibody has an optimal dilution.

Most formalin fixed tissue requires an antigen retrieval step before immunohistochemical staining can proceed. This is due to the formation of methelyne bridges during fixation, which cross- link proteins and therefore masks antigenic sites. The two methods of antigen retrieval are heat-mediated antigen retrieval and enzymatic antigen retreival; in this experiment heat mediated antigen retrieval method was used; as described by previous reports (Greiner et al., 2005; Ghushima et al., 2010; Shinmura et al., 2011). This method is performed using a pressure cooker, microwave, or hot plate. In our experiment, IHC was performed using microwave. AID antigen was calibrated by using different levels of power at different timing; at Mid power (10 min, 20 min), at Mid High power (10 min, 20min), and at High power (10, 15, 20 min), and the perfect results obtained were at High power for 15 min. For performing heat induced antigen retrieval proper buffer should be calibrated, citrate buffer (pH=6) was used for this project as described in previous report (Greiner et al., 2005).

In this project, monoclonal Anti-Human\Mouse Activation Induced Cytidine Deaminase (AID) was used, it recognizes human and mouse AID. Calibration for the accurate dilution of the AID Ab was done by using different buffer concentrations. We have used Ab:cass block concentration of 1:50, 1:100, 1:200. The optimum staining result was obtained by the dilution 1:50. Cass block is a universal blocking agent for reducing nonspecific background staining in immunolableing techniques, and works well as a diluting reagent for primary Abs. Slides were incubated with primary Ab for one hour at room T, and overnight at 4 C, the best results obtained by overnight incubation.

- Slides were treated with hydrogen peroxide (H_2O_2), for blocking endogenous peroxidase activity which may lead to false positive results, since we are using DAB as a substrate for peroxidase enzyme labeled on the secondary Ab. We have used control slides at different (H_2O_2) timing; 10 min and 15 but the results were not different.

- The secondary Ab used is labled with horse radish peroxidase (HRP), and DAB substrate chromagen is suitable for use with HRP detection system; it enables the antibody antigen complex to be viewed under the light microscope, this occurs because DAB acts as an electron donor in the presence of the HRP, DAB gets reduced and the color change to brown. Slides were incubated with DAB at different timing; 5 min, 10 min, 15 min, 20 min, 25 min, and the best results obtained at 25 min.

- Evaluation of AID protein expression, within the slides was supervised by the pathologist Dr. Riyad Shreim, and the intensity of cytoplasmic expression classified according to the three grade scale (0: no staining; 1+: weak positive staining, +2 strong positive staining) (Gushima et al., 2011).

2.2.5. Statistical Analysis:

Statistical analysis was performed to find if there is a significant correlation between AID protein expression and clinic-pathological data of the cases. **Pearson's chi-squared test** was used, and converted to P-value. P-value <0.05 was considered to be statically significant.

Chapter Three: Results

3.1. Study Samples:

The general clinical and pathological data of LC cases are summarized in Table 4; this includes age, gender, smoking status, LC type, and tumor grade. These data were organized randomly.

As shown in Table 4, IHC was performed for 58 LC cases and one normal lung sample, and for further confirmation, a whole set of slides (8 cases) chosen randomly, were repeated IHC with similar results.

Table 4: Clinico-pathological Data &AID Expression for the LC Cases.

S. #	Block #	Age	Sex	Smoking status	LC type	Tumor grade	AID Exp.
1.1	1655-09 IE	50	M	-	Squamous cc	Moderately d.	**+1**
2.	728-07	51	M	S	Squamous cc	Moderately d.	**0**
3.	1873-08	53	M	-	Squamous cc	Moderately d.	**+1**
4.	1049-07 H	50	M	S	Adenocarcinoma	Moderately d.	**0**
5.	968-09	39	F	NS	Squamous cc	Moderately d.	**0**
6.	59-09	68	F	NS	Adenocarcinoma	Moderately d.	**0**
7.	1304-08	74	M	S	Squamous cc	Moderately d.	**0**
8.	822-04	42	M	S	Adenocarcinoma.	Moderately d.	**0**
9.	835-08	78	M	S	Squamous cc	Moderately d.	**0**
10.	2047-09	63	M	-	Squamous cc	Poorly d.	**0**
11.	1088-07	61	M	S	Squamous cc	Poorly d.	**0**
12.	743-10	60	M	NS	Squamous cc	Poorly d.	**0**
13.	1432-01	76	M	NS	Adenocarcinoma	Moderately d.	**0**

14.	33-06 II	38	M	S	Adenocarcinoma	Moderately d.	**0**
15.	1046-03	49	M	S	Adenocarcinoma	Moderately d.	**0**
16.	48-08	75	M	S	Adenocarcinoma	Moderately d.	**0**
17.	596-10 II	78	M	-	Adenocarcinoma	Poorly d.	**0**
18.	2764-01	69	M	NS	Squamous cc	Moderately d.	**0**
19.	1995-09 IJ	61	M	-	Squamous cc	Moderately d.	**0**
20.	585-10 I	75	M	S	Adenocarcinoma	Moderately d.	**0**
21.	517-05	40	F	NS	Squamous cc	Poorly d.	**0**
22.	871-07	46	M	S	Adenocarcinoma	Moderately d.	**0**
23.	728-03 IIA	63	M	S	Adenocarcinoma	Poorly d.	**0**
24.	2185-11 II	69	F	-	Adenocarcinoma	Poorly d.	**0**
25.	1361-11 I	64	M	-	Adenocarcinoma	Poorly d.	**0**
26.	1848-11 I	65	M	S	Squamous cc	Moderately d.	**0**
27.	1245-11 I	51	M	-	Adenocarcinoma	Moderately d.	**0**
28.	1673-11	50	M	-	Adenocarcinoma	Moderately d.	**0**
29.	2331-11 I	52	M	S	Squamous cc	Poorly d.	**0**
30.	2449-11 IE	65	M	-	Squamous cc	Poorly d.	**0**
31.	1188-11	68	M	-	Squamous cc	Poorly d.	**0**
32.	2449-11 IC	65	M	-	Squamous cc	Poorly d.	**+1**
33.	1245-11 IIA	51	M	-	Adenocarcinoma	Moderately d.	**0**
34.	2185-11 I	69	F	-	Adenocarcinoma	Poorly d.	**+1**
35.	2061-11	62	M	-	SCLC	Poorly d.	**+1**
36.	2861-10 A	50	M	-	Squamous cc	Well d.	**0**
37.	1698-10	78	M	-	Adenocarcinoma	Poorly d.	**+1**
38.	1700-10 I	80	M	-	Squamous cc	Poorly d.	**0**
39.	596-10 I	78	M	-	Adenocarcinoma	Poorly d.	**0**
40.	1245-10	61	F	-	SCLC	Poorly d.	**+1**
41.	1310-10	73	F	-	Squamous cc	Moderately d.	**0**
42.	1423-10	57	M	-	Squamous cc	Moderately d.	**0**
43.	1913-10	42	F	-	Adenocarcinoma	Moderately d.	**0**
44.	2837-10 C	59	F	-	Adenocarcinoma	Poorly d.	**0**
45.	1359-10 A	70	M	-	Squamous cc	Poorly d.	**0**

46.	1636-10 II	66	M	-	Squamous cc	Moderately d.	**0**
47.	1479-08	47	M	-	Squamous cc	Moderately d.	**+1**
48.	1726-092	34	M	-	SCLC	Poorly d.	**+1**
49.	1247-08	42	F	-	Adenocarcinoma	Poorly d.	**+1**
50.	1995-9 IA	61	M	-	Squamous cc	Moderately d.	**0**
51.	761-09	60	M	S	Squamous cc	Moderately d.	**0**
52.	1230-08	62	M	-	Adenocarcinoma	Poorly d.	**0**
53.	1655-9 IB	50	M	-	Squamous cc	Moderately d.	**+1**
54.	1304-8 E	74	M	S	Squamous cc	Poorly d.	**+1**
55.	1639-8	62	M	-	Squamous cc	Moderately d.	**0**
56.	1995-9 II	61	M	-	Squamous cc	Moderately d.	**0**
57.	1306-8	74	M	-	Squamous cc	Moderately d.	**+1**
58.	1647-09	69	F	-	Adenocarcinoma	Moderately d.	**0**
*	Normal L.	-	-	-	-	-	**0**

M: male, F: female, S: smoker, NS: nonsmoker.

3.2 Summary of the Clinico-Pathological Data of the LC Cases:

As shown in table 5; 81% of the patients were males and 19% were females. The age of the patient's ranges between 34 years and 80 years, the average was (55 years). The table also shows the smoking status of some patients; 16 patients were smokers, and 6 patients were nonsmokers.

As for LC staging the table shows that 95% were NSCLC [among them (44% adenocarcinoma, and 56% squamous cell carcinoma], and 5% were SCLC, this is agreement with other studies showing that NSCLC is the most common LC type. As for tumor grade, 41% were poorly differentiated, 57% were moderately differentiated, and 2% were well differentiated.

Table 5: Summary of the Clinico-pathological Data of LC Cases.

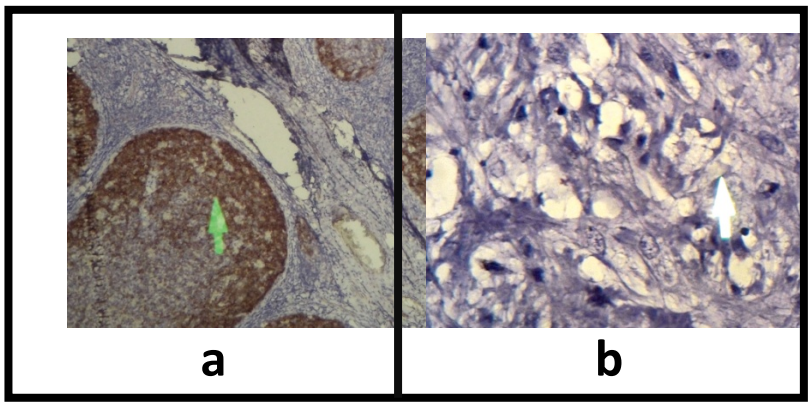
Patient characteristics:		**Frequency (%)**
Gender	*Male* *Female*	47 (81%) 11 (19%)
Age	*Range* *>=60* *<60* *Average*	34-80 36 (62%) 22 (38%) 55
Smoking:	***Among Documented:***	

	Smoker *Non smoker*	16 6
LC type:	***NSCLC:*** *Squa* *Adeno* ***SCLC:***	55 (95%) 31 (56% of NSCLC) 24 (44% of NSCLC) 3 (5%)
Tumor Grade:	*Poor d* *Mod d* *Well d*	24 (41%) 33 (57%) 1 (2%)

3.3 IHC Evaluation and Statistical Analysis of the LC cases:

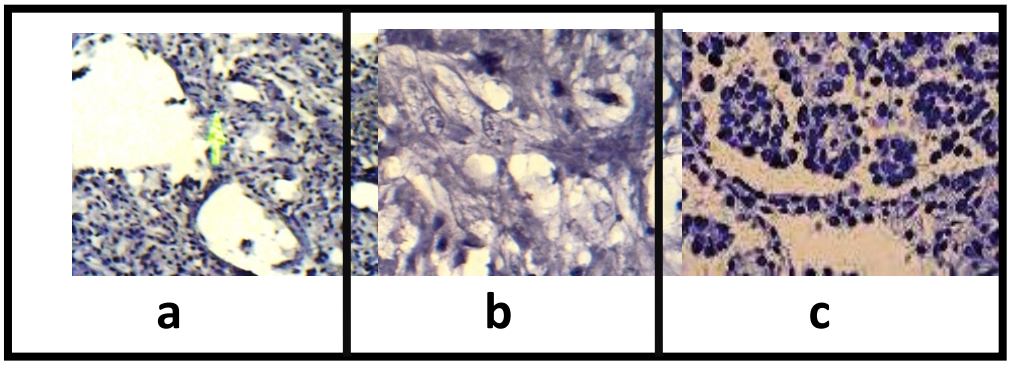
Positive controls (tonsils tissue sections), were stained in brown with cytoplasmic localization (Figure 6-a), and negative controls were not stained (Figure 6-b).

Figure 6: Cytoplasmic AID Expression in Positive Control (a), and Negative Control (b), at 10x magnification.



AID protein expression was detected in 22.4% (13 of 58) of cases with cytoplasmic localization, No expression was revealed in normal lung tissue (Figure 7-a) or in 77.5% (45 of 58) of the cases including squamous cell carcinoma and adenocarcinoma (Figure 7-b, 7-c, respectively).

Figure 7: Negative AID Expression in Normal lung Tissue (a), Squamous cc (b), and Adenocarcinoma (c), at 10x magnification.



As shown in table 6, 18% (10 of 55) of NSCLC, and 100% (3 of 3) of SCLC (Figure 8-a) were positive cases. Among NSCLC, 23% (7 of 31) of squamous cell carcinoma (Figure 8-b), and 13% (3 of 24) of adenocarcinoma (Figure 8-c) were positive. No significant difference was found between LC types (P=0.9985), or subtypes (P=0.5621, not shown in the table).

Figure 8: Positive AID Expression in SCLC (a), Squamous cc (b), Adenocarcinoma (c), at 10x magnification.

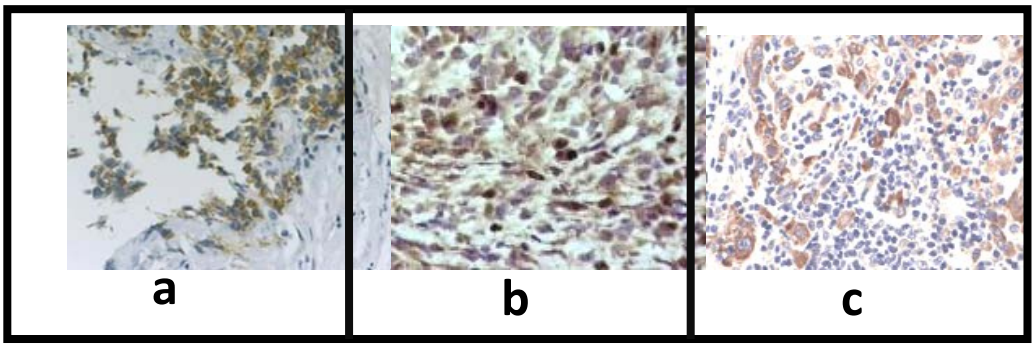


Table 6 shows that; 15%, and 33% of , moderately, and poorly differentiated; show AID protein positive, respectively, and no AID protein expression in well differentiated cases. No significant difference was found between LC tumor grade (P=0.7240). The table also shows that; 21% of males and 27% of females were positive cases, and no significant difference was found between gender (P=0.4137), and the table shows that 19% of the positive cases were over or equal 60 years, and 27% were below 60 years, and no significant difference was found between the two groups of age (P= 0.4848). Since most of the cases did not have complete clinical data concerning smoking status, percentage among positive cases or correlation with AID protein expression could not be detected.

Table 6: Correlation between AID Protein Expression and Clinico-pathological Parameters.

Variable\AID	0	+1	Total	% of AID positive cases	$\chi 2$	P value
Gender					0.668	0.4137
M	37	10	47	21%		
F	8	3	11	27%		
Age					0.488	0.4848
>=60	29	7	36	19%		
<60	16	6	22	27%		
LC type					0.003	0.9985
NSCLC	45	10	55	18%		
Adenocarcinoma	21	3	24	13%		
Squamous cc	24	7	31	23%		
SCLC	0	3	3	100%		
Tumor grade					0.646	0.7240
Well d.	1	0	1	0%		
Moderately d	28	5	33	15%		
Poorly d.	16	8	24	33%		

- $\chi 2$: Chi square test.
- P value ≤0.05, AID protein expression versus clinico-pathological parameters.

Discussion

Activation induced cytidine deaminase (AID), is expressed in activated B cells and is involved in antibody diversification by inducing mutations immunoglobulin genes (Revy et al., 2000). AID has been shown to attack non-immunoglobulin genes and induce mutations broadly throughout the genome in B cells (Nogaoka et al., 2010). Aberrant AID expression can induce mutations in fibroblasts, and the constitutive expression of AID in mice causes not only lymphomas, but also cancers of other organs including the lung, suggesting that ectopically expressed AID has an oncogenic potential via its mutagenic activity (Okazaki 2003). In accordance several studies has been reported that AID is aberrantly expressed in several cancer types such as gastric carcinoma (Matsumoto et al., 2007), hepatocellular carcinoma (Takai 2009), lymphoma and leukemia (Klemm et al., 2009), Hodgkin lymphoma (Greiner et al., 2005), esophageal squamous cell carcinoma (Zhang et al., 2011), colitis associated colorectal cancer (Endo et al., 2008), and lung cancer (Shinmura et al., 2011).

A recent study by Shinmura et al 2011, reported for the first time, the aberrant AID expression in human LC. This study showed aberrant mRNA expression of AID in 29% (5 of 17) of the LC cell lines and 31% (16 of 51) of the primary LC. However, this aberrant AID mRNA expression didn't show any association with the clinico-pathological factors such as age, gender, smoking history, tumor stage, or tumor histology. As for AID protein expression, analysis revealed that AID protein expression was mostly cytoplasmic with a small portion of AID localized at the centrosomes (Shimura et al., 2011). No association was detected between AID protein expression level and the clinicopathological factors such as gender or tumor histology. Furthermore, an association was found between the AID protein expression level and the p53 mutation status in an analysis of 129 primary LC. When LC cases were divided into NSCLC and SCLC, an association between the p53 mutation status and AID protein expression level was also detected in NSCLC (Shinmura et al., 2011).

In the present study, we examined the expression of AID protein in LC tissue sections derived from Palestinian patients' paraffin embedded blocks. AID signal was mainly observed in the cytoplasm in accordance with previous studies (Shinmura et al., 2011; Kou et al., 2006; Endo et al., 2008; Zhang et al., 2011). Results revealed that AID protein is

overexpressed in 21% of LC cases. In particular 20% (10 of 50) of NSCLC and 100% (3 of 3) of SCLC express AID protein. Among NSCLC 29% of squamous cell carcinoma, 13% of adenocarcinoma, and 0% of large cell carcinoma express AID protein. Our results are consistent with the study of Shinmura et al showing AID protein expression in 35.7% (46 of 129) of the cases (Shinmura et al., 2011). Interestingly, SCLC cases examined by Shinmura showed expression in 40.0% (4 of 10) of the cases while our results in Palestinian cases of SCLC showed 100% (3 of 3). Protein level of AID was not detected in normal lung tissue, because AID is not expressed under physiological conditions in normal tissues other than tonsils or lymph nodes; where it functions in CSR and SHM (Meyers et al., 2011). In our results correlation between AID protein expression and clinic-pathological parameters; age, sex, LC type, tumor grade, was not detected.

To explain the tumorgenic activity of AID among these patients, other experiments should be performed, examining regulation of AID and its tumorgenic effect inducing mutations in tumor suppressor genes or oncogenes. AID is regulated by several factors; AID promoter includes sites for the transcription factors NFkB, STAT6, HoxC4, Sp1 and Pax5 (Delker 2009). Several factors can influence and trigger NFkB, which is a major activator of AID expression. As a major cell survival signal, NFkB is involved in multiple-steps in carcinogenesis (Chen et al., 2012). Recent studies showed that NF-kappaB is constitutively activated in a variety of solid tumors, including prostate, breast, cervical, pancreatic and lung cancer (Chen et al., 2012). Tumor samples obtained from lung cancer patients showed high levels of NF-kappaB activation in both (SCLC) and (NSCLC) (Chen et al., 2012). A large body of evidence suggests that inflammation plays an important role in lung cancer development (Chen et al., 2012). Exposure to asbestos is associated with the development of pulmonary fibrosis, and lung carcinogenesis (Janseen et al., 1995). Several studies showed that NF-kB is induced by asbestos, and this is a key event in regulation of multiple genes involved in the pathogenesis of asbestos related LC (Janseen et al., 1995).

Cigarette smoke (CS) is a common cause of chronic lung inflammation (Anand et al., 2003). Myeloid cells (mainly macrophages) are the major source of inflammatory cytokines for cancer promotion and progression (Anand et al., 2003). NF-kappaB is a major signal in mediating cytokine synthesis and secretion from myeloid cells. Thus, it is suggested that NF-kappaB in myeloid cells promotes lung cancer mainly through mediating inflammatory cytokines secretion to establish a cancer-prone inflammatory microenvironment (Anand et al., 2003). In addition, NF-kappaB in epithelial cells also

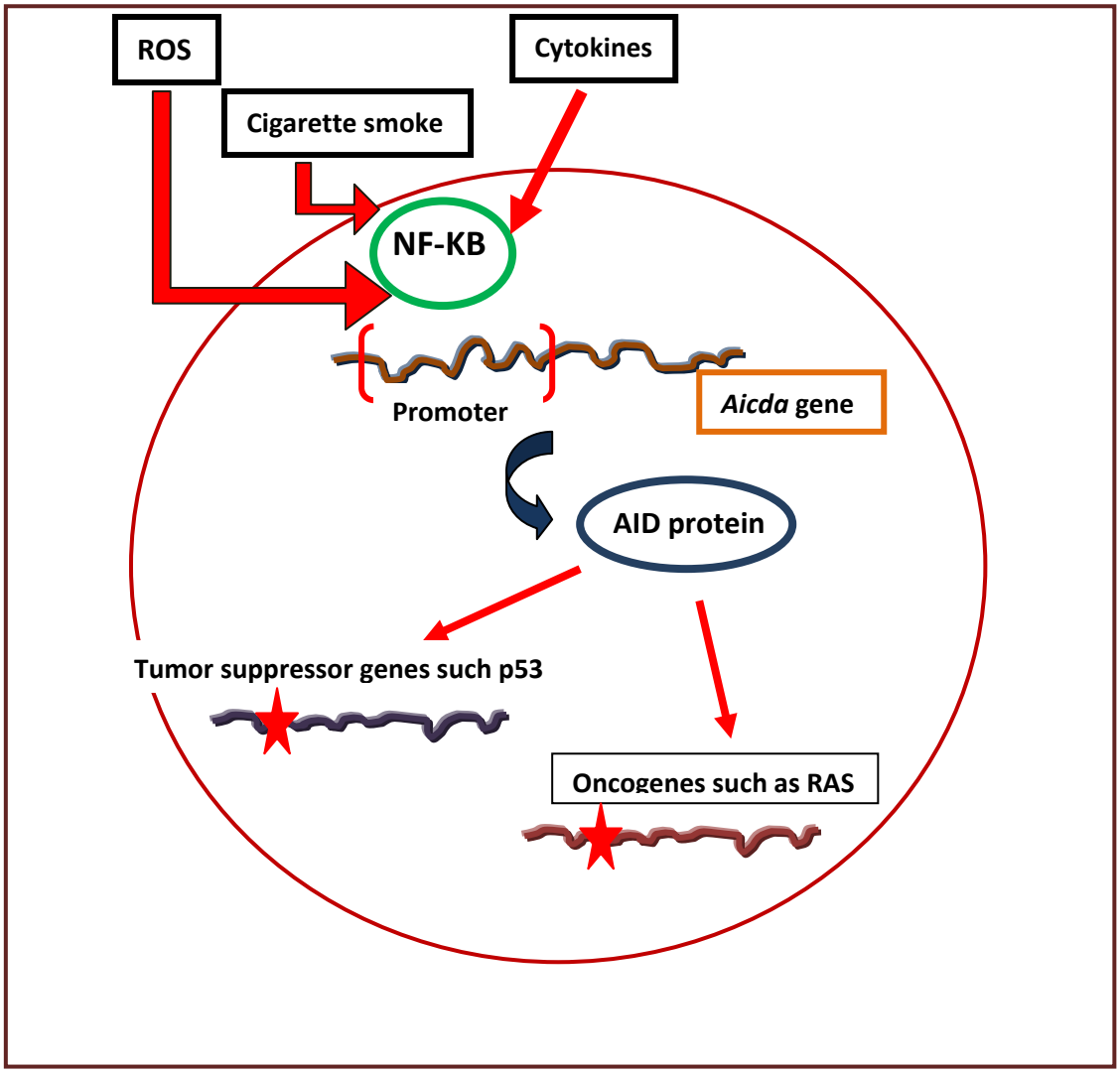
plays a lung cancer-promoting role (Anand et al., 2003). NF-kappaB has been shown to be activated by cigarette smoke (CS) in a panel of NSCLC cell lines (Anand et al., 2003). Because NF-kappaB is persistently activated by CS in the lung epithelial cells far before tumor formation, it is likely that this cell survival signal promotes mutant cells to proliferate and to escape apoptosis in the early phase of lung cancer development (Chen et al., 2012). Further studies on this issue should provide us with more information regarding possible activation of AID by NFkB in LC.

Several studies showed that AID induces genomic instability and tumorgenesis by inserting mutations in tumor related genes in both lymphoid and non-lymphoid organs. Study on murine cell lines showed several genes e.g. myc, bc16, pim1 pax5 and others are deaminated by AID (liu et al., 2008). AID-induced p53 mutations were reported in hepatocarcinogenesis (Takai et al., 2009), gastric epithelial cells (Matsumoto et al., 2007), B cell chronic leukemia (Malickova et al., 2008), and LC (Shinmura et al., 2011).

Mutational activation of oncogenes and inactivation of tumor suppressor genes and subsequent increased genetic instability are major genetic events in lung carcinogenesis. KRAS mutations occur primarly in adenocarcinoma and are seen at a much lower frequency in non-smokers (5%) than in smokers (30%). P53, RB1 and P16 mutations and inactivation have the same frequency in adenocarcinoma as in squamous cell carcinoma. Squamous cell carcinomas show high frequency of p53 mutations. SCLC shows aggressive behavior and poor prognosis, and has a strong relationship to cigarette smoking; only about 1% occurs in non-smokers. P53 and RB1 tumor suppressor genes are frequently mutated in 50% to 80% and 80% to 100% of SCLC, respectively (Robbins and Cotran, 2010)

In this study a possible suggestion for AID activation in these LC cases is that cigarette smoke may induce NFkB and this in turn binds to the promoter of AID and induces its expression. Activated AID induces mutations in tumor suppressor genes and oncogenes. Further studies are needed to verify this hypothesis; figure 9 suggests a possible mechanism for the pathway of AID induction in LC cells.

Figure 9: Proposed Mechanism of AID Induction in LC through NF-kB Activation.



In conclusion, our findings show the aberrant expression of AID may be involved in a subset of LC patients, with variations from one type to another. No correlation was detected between AID expression and age, gender, LC type or tumor grade. More studies are needed to shed the light on AID induction, mechanism of action, regulation and possible targets within LC.

Recommendations

To enable the researcher to study AID expression and correlation with clinico-pathological data provided in a large cohort of patients, more cases of LC types and subtypes will be examined for AID expression.

A major problem in clinical research in Palestine is the insufficient clinical data in the patient's files which makes the statistical analysis of the data for clinical research inadequate.

IHC technique will performed with other techniques for further confirmation, such as RT-PCR, western blott, and this requires fresh tissue to be obtained from patients, so cooperation with researchers at the hospitals is of great importance.

I recommend in the future that the oncologist, pathologist and molecular biologist, contribute from the start of the project to obtain samples and full clinical information needed for the project.

References

Abdeen H. (2006) Chronic diseases in Palestine: the rising tide. Israeli-Palestinian Public Health Magazine (Bridges) 2(3).

Anand P, Kunnumakara A, Sundaram C, Harikumar K, Tharakan S, Lai O, Sung, and Aggrawal B. (2008). Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical Research 25(9):2097-116.

Avwioro G. (2011). Histochemical uses of hematoxylene - A review. JPCS 1:26-34

Beckels MA, Spiro SG, Colice GL, and Rudd RM. (2003) Initial evaluation of the patient with lung cancer. Chest Journal 123(1):97S-104S.

Biesalski HK, Mesquita BB, Chesson A, Chytil F, Grimble R, Hermus RJJ, Kohrle J, Lotan R, Norpoth K, Pastorino U, and Thurnham D. (1998) European consensus statement of lung cancer: Risk factors and prevention. Ca J Clin. 48:167-176.

Chen W, Li Z, Bai L, and Lin Y. (2012). NF-kappaB, a mediator for lung carcinogenesis and a target for lung cancer prevention and therapy. NIH 16:1172-1185.

Chiba T, Marusawa H, Ushijima T. (2012) Inflammation-associated cancer development in digestive organs: mechanisms and roles for genetic and epigenetic modulation. Gastroenteriology 143:550-63.

Collins LG, Haines C, Perkel R, and Enck RE. (2007) Lung Cancer diagnosis and management. American Family Physician 75(1):56-63.

Conticello SG. (2008) The AID/APOBEC family of nucleic acid mutators. Genome Biology 9(6):229.1-299.10

Dedeoglu F, Horwitz B, Chaudhuri J, Alt FW, Geha RS. (2004) Induction of activation-induced cytidine deaminase gene expression by IL-4 and CD40 ligation is dependent on STAT6 and NF-ĸB. Int Immunol, 16:395-404.

Delker RK, Fugmann SD, and Papavasiliou FN. (2009) A coming-of-age story: activation induced cytidine deaminase turns 10. National Institute of Public Health 10(11):1147-53.

Devereux RT, Taylor JA, and Barrett CJ. (1996) Molecular mechanisms of lung cancer: interaction of environmental and genetic factors. Chest 109(3):14S-19S

Ebert W, Dienemann H, Fateh-Moghadam A, Scheulen M, Konietzko N, Schleich T, and Bombardieri E. (1994) Cytokeratin 19 fragment CYFRA 21-1 compared with carcinoembryonic antigen, squamous cell carcinoma antigen and neuron specific enolase in lung cancer. Results of an international multicentre study. Eur J Clin Biochem 32:189-99.

Endo Y, Marusawa H, Kinoshita K, Morisawa T, Sakurai T, Okazaki IM, Watashi K, Shimonoto K, Honjo T, Chiba T (2007) Expression of human activation-induced cytidine deaminase in human hepatocyte via NF-κB signaling. Oncogene 26:5587-95.

Endo Y, Marusawa H, Kou T, Nakase H, Fujii S, Fujimori T, Kinoshita K, Honjo T, and Chiba T. (2008). Activation induced cytidine deaminase links between inflammation and the development of colitis-associated colorectal cancers. Gastroenerology 135:889-98.

Gilligan D, Rintoul R. (2007) Your guide to lung cancer. The royal society of medicine 1-36.

Godwin Avwioro. (2011) Histochemical Uses of Haematoxylin - A Review. JPCS 1:24-34

Greiner A, Tobollik S, Buettener M, Jungnickel B, Hermann K, Kremmer E, and Neidobitek G. (2005). Differential expression of activation induced cytidine deaminase (AID) in nodular lymphocyte-predominant and classical Hodgkin lymphoma. J Pathol 205: 541-547.

Herbst RS, Heymach JV, and Lippman SM. (2008) Molecular origins of cancer: Lung cancer. N ENGL J MED 359(13):1367-80.

Holmes RK, Malim MH, Bioshop KN. (2007) APOBEC-mediated viral restriction: not simply editing? Trends in Biochemical Sciences 32(3): 118-28.

Honjo T, Kinoshita K, and Muramatsu M. (2002) Molecular mechanisms of class switch recombination: linkage with somatic hypermutation. Ann. Rev. Immunol. 20:165-96.

Janseen Y, Barchowsky A, Traedwell M, Driscoll K, Moosman B. (1995). Asbestos induce nuclear kB(NF-kB) DNA binding activity and NF-kB-dependent gene expression in tracheal epithelial cells. Proc. Natl. Acad. Sci. USA 92:8458-62.

Jemal A, Bray F, Melissa M, Ferlay J, Ward E, Forman D. (2011) Global Cancer Statistics. CA CANCER J CLIN;61:69–90.

Klemm L, Duy C, Iacobucci I, Kushen S, von Levetzow G, Feldhahn N. (2009) the B cell mutator AID promotes B lymphoid blast crisis and drug resistance in chronic myeloid leukemia. Cancer Cell 16:232-45.

Kou T, Marusawa H, Kinoshita K, Endo Y, Okazaki H, Ueda Y, Kodama Y, Hega H, Chiba T (2006) Expression of activation-induced cytidine deaminase in human hepatocyte during hepatocarcinogenesis. Int J Cancer 120:469-76.

Kuraoka M, Holl TM, Liao D, Womble M, Cain DW, Reynolds AE, , Kelsoe G. (2011) Activation-induced cytidine deaminase mediates central tolerance in B cells. Proceedings of the National Academy of Sciences of the United States of America. 108:11560–5.

Larijani M, Martin A. (2012) The biochemistry of activation-induced cytidine deaminase and its physiological functions. Seminars in Immunology 24:255-63.

Lee-Theilen M, and Chaudhuri J. (2010) The mutator activation-induced cytidine deaminase is essential for immunoglobulin diversification but can be determinal in other settings. A new comprehensive analysis investigates how its gene expression is regulated. Nature Immunology 11(2):107-9.

Liu M, Duke J, Richer D, Vinuesa C, Goodnow C, Kleinstein S and Schatz D. (2008) Two levels of protection for the B cell genome during somatic hypermutation. Nature 451:841-6.

Malicikova J, Smardova J, Pekova S, Cejkova S, Kotaskova J, Tichy B, Francova H, Doubek M, Brychtova Y, Janek D, Pospisilova S, Mayer J, Dvorakova D, and Trbusek M. (2008) Identification of somatic hypermutation in the p53 gene in B-cell chronic lymphatic leukemia. Mol. Immunol. 45:1525-9.

Marusawa H and Chiba T. (2010) Helicobacter pylori-induced activation-induced cytidine deaminase expression and carcinogenesis. Current Opinion in Immunology 22:442-7.

Marusawa H, Hijikata M, Chiba T, Shimotono K. (1999) Hepatitis C virus core protein inhibits Fas- and tumor necrosis factor alpha-mediated apoptosis via NF-κB activation. J Virol 73:4713-20.

Matsumoto Y, Marusawa H, Kinoshita K, Endo Y, Kou T, Morisawa T, Azuma T, Okazaki IM, Honjo T, and Chiba T. (2007) Helicobacter pylori infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. Nature Medicine 13(4):470-6.

Maul R, and Gearhart P. (2009) Women autoimmunity, and cancer: a dangerous liaison between estrogen and activation induced deaminase. JEM 206:11-3.

Meyers G, Ng YS, Bannock JM, Lavoie A, Walter JE, Notarangelo LD, Kilic SS, Aksu G, Debre M, Rieux-Laucat F, Conley ME, Cunningham-R C, Durandy A, Meffre E. (2011) Activation-induced cytidine deaminase (AID) is required for B-cell tolerance in humans. Proceedings of the National Academy of Sciences of the United States of America 108:11554–9.

Moldenhauer G, Popov S, Wotschke B, Brüderlein S, Riedl P, Fissolo N, Schirmbeck R, Ritz O, Möller P and Leithäuser F. (2006) AID expression identifies interfollicular large B cells as putative precursors of mature B-cell malignancies. Blood Journal 107: 2470-3.

Mountain CF. (2008) Revisions in the international system for staging lung cancer. American College of Chest Physicians 111(6):1710-7.

Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, Davidson NO, and Honjo T. (1999) Specific expression of activation-induced cytidine damiinase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. The Journal of Biological Chemistry 274(26):18470-6.

Nagaoka H, Tran TH, Kobayashi M, Aida M, and Honjo T. (2010) Preventing AID, a physiological mutator, from deleterious activation: regulation of the genomic instability that is associated with antibody diversity. International Immunology 22(4):227-35.

Okazaki IM, Hiai H, Kakazu N, Yamada S, Muramatsu M, Kinoshita K, and Honjo T. (2003) Constitutive expression of AID leads to tumorigenesis. J. Exp. Med 197(9):1173-81.

Palestinian Ministry of Health MOH. (2002) First report on Oncology in Palestine by Health Management Information System (HMIS)-MOH. Health Inforum 1(15).

Pandya KJ, Brahmer JR, and Hidalgo M. (2007) Lung cancer translational and emerging therapies. Informa Health Care New Yourk USA.

Pauklin S, Sernandez IV, Bachmann G, Ramiro AR, and Peterson-Mahrt SK. (2009) Estrogen directly activates AID transcription and function. JEM 206(1):99-111.

Porta RR, Crowley JJ, and Goldstraw P. (2009) The revised TNM staging system for lung cancer. Ann Thorac Cardiovasc Surg 15(1):331-8.

Ramos-Vara JA. (2005) Technical aspects of immunohistochemistry. Vet Pathol. 42(4):405-26.

Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, Catalan N, Forveille M, Dufourcq-Labelouse R, Gennery A, Tezcan I, Ersoy F, Kayserili H, Ugazio AG, Brousse N, Muramatsu M, Notarangelo LD, Kinoshita K, Honjo T, Fischer A, Durandy A. (2000) Activation induced-cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). Cell 102:565-75.

Robbiani DF, Bunting S, Feldhahn N, Bothmer A, Camps J, Deroubaix S, McBride KM, Klein IA, Stone G, Eisenreich TR, Ried T, Nussenzweig A, Nussenzweig MC. (2009) AID produces DNA double-strand breaks in non-Ig genes and mature B cell lymphomas with reciprocal chromosome translocations. Mol Cell. Nov 25;36(4):631-41.

Salgia R, Skarin AT. (2012) Molecular abnormalities in Lung cancer. Journal of Clinical Oncology 16(3):1207-17.

Schalhorn A, Fuerst H, Stieber P. (2001) Tumor markers in lung cancer. J lab Med 25:353-61.

Shibayama T, Ueoka H, Nishii K, Kiura K, Tabata M, Miyatake K, Kitajima T, and Harada M. (2001) Complementary roles of pro-gastrin-releasing peptide (proGRP) and neuron

specific enolase (NES) in diagnosis and prognosis of small-cell lung cancer (SCLC). Lung Cancer 32:61-9.

Shinmura K, Igarashi H, Goto M, Tao H, Yamada H, Matsuura S, Tajima M, Matsuda T, Yamane A, Funai, K, Tanahashi M, Niwa H, Ogawa H and Sugimura H. (2011) Aberrant expression and mutation induced activity of AID in human lung cancer. Ann Surg Oncol 18:2084-92.

Sozzi G, Veronese M, Negrini M, Baffa R, Cotticelli MG, Inoue H, Tornielli S, Pilotti S, De Gregorio L, Pastorino U, Pierotti MA, Ohta M, Huebner K, Croce CM.(1996) The FHIT gene at 3p14.2 is abnormal in Lung Cancer. Cell 85:17-26.

Stieber P, Hatz R, Holddenrieder S, Molina R, Nap M, Pawel J, Schalhorn A, Schneider J, and Yamaguchi K. National academy of clinical biochemistry guidelines for the use of tumor markers in lung cancer. NACB Section 3b

Takai A, Toyoshima T, Uemura M, Kitawaki Y, Marusawa H, Hiai H, Yamada S, Okazaki IM, Honjo T, Chiba T, and Kinoshita K. (2009) A novel mouse model of hepatocarcinogenesis triggered by AID causing deleterious p53 mutations. Oncogene 28:469-78.

Takaishi S, and Wang TC. (2007) Providing AID to p53 mutagenesis. Nature Medicine 13(4):404-6.

Terada T. (2012) An immunohistochmical and molecular genetic analysis of KIT and PDGFRA in small cell Lung carcinoma in Japanease. Int J Clin Pathol 5(4):331-8.

Travis WD. (2002) Pathology of Lung Cancer. Clinics in Chest Medicine. 23(1):65-81.

Xu Z, Pone EJ, Al-Qahtani A, Park SR, Zan H, Casali P (2007) Regulation of aicda expression and AID activity: Relevance to somatic hypermutation and class switch DNA recombination. Crit Rev Immunol 27(4): 367–97.

Yamaguchi K, Ayoyagi K, Urakami K, Fukutani T, Maki N, Yamamoto S, Otsubo K, Miyake Y, and Kodama T. (1995) Enzyme-linked immunosorbent assay of pro-gastrin-releasing peptide for small cell lung cancer patients in comparison with neuron-specific enolase measurement. Jpn J Cancer Res 86:698-705.

Yokota J, Kohno T. (2004) Molecular footprints of human lung cancer progression. Cancer Sci. 95(3):197-204.

You M, Candrian U, Maronopot RR, Stoner GD, and Anderson MW (1989). Activation of the K-ras proto-oncogene in spontaneously occurring and chemically induced lung tumors of the strain A mouse. Proc Natl Acad Sci USA 86:3070-4.

Zhang L, Hu S, Korteweg C, Chen Z, Qiu Y, Su M, Gu J. (2012). Expression of immunoglobulin G in esophageal squamous cell carcinomas and its association with tumor grade and Ki67. Human Pathology 43: 423-32.

فحص مستوى البروتين (Activation induced-cytidine Deaminase) عند مرضى سرطان الرئة

اعداد الطالبة: كوثر علي القضماني

اشراف الدكتورة: رولا عبد السلام عبد الغني

سرطان الرئة هو من اكثر انواع السرطان الشائعة في العالم, فهو يصيب اكثر من مليون مريض كل عام, وهو السبب الرئيسي للوفيات المتعلقة بالاصابة بمرض السرطان, في فلسطين. وبناء على احصائيات وزارة الصحة لعام 2006, فان سرطان الرئة يحتل المرتبة الرابعة من حيث عدد الاشخاص المصابين, و يحتل المرتبة الاولى من حيث عدد الوفيات في المجتمع ككل.

البروتين (AID), من عائلة انزيمات ال (APOBEC) يلعب دورا مهما في عملية المناعة و بناء الاجسام المضادة. و بالرغم من ان الاهداف الفسيولوجية لهذا البروتين هي الجينات المتعلقة بالاجسام المضادة في جهاز المناعة, الا انه قد يستهدف جينات اخرى تلعب دورا مهما في نشأة السرطان. وقد اثبتت دراسات كثيرة العلاقة الكبيرة بين ارتفاع مستوى هذا البروتين في الخلايا و انواع متعددة من السرطان, كسرطان المعدة, الثدي, الامعاء و الكبد و و التهاب القولون التقرحي و غيرها.

الهدف من هذه الدراسة, هو فحص مستوى هذا البروتين في عينات لمرضى سرطان الرئة, و لتحقيق هذه الاهداف قمنا بجمع عينات 58 مريض بسرطان الرئة من مستشفى المقاصد الخيري بالقدس, خلال السنوات العشر الاخيرة من عام 2000-2011 وقمنا بفحص مستوى البروتين (AID) من خلال تجربة ال Immunohistochemistry.

وقد اظهرت النتائج ان البروتين AID موجود عند 21% من مرضى سرطان الرئة الذين قمنا بفحصهم. و انه موجود بنسبة 25% عند المرضى من نوع (NSCLC), و بنسبة 100% عند نوع (SCLC). و اظهرت النتائج الاحصائية عدم و جود علاقة بين مستوى هذا البروتين مع المتغيرات الطبية للمرضى مثل العمر و الجنس و نوع سرطان الرئة و مستوى المرض و هذه النتائج تفتح ابواب جديدة للبحث العلمي في مجال سرطان الرئة لدى المرضى الفلسطينيين.