Study of the sensitivity of lung cancer cell lines to TRAIL-induced apoptosis

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ABSTRACT

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Title of thesis: Study of the sensitivity of lung cancer cell lines to TRAIL-induced apoptosis

Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is an apoptogenic cytokine with great tumor specificity and has been recently considered as the magic bullet in the treatment of cancer. Recently, TRAIL-DR4 and TRAIL-DR5 directed monoclonal antibodies showed anti-tumor effect against a panel of non-small cell lung cancer (NSCLC) cell lines in vitro with different levels of sensitivity to TRAIL-induced apoptosis. The present study was aimed to investigate possible reasons for TRAIL resistance in NSCLC cell lines by searching for mutations at TRAIL receptors levels using single strand conformation polymorphism (SSCP) and at the downstream signaling pathway through determination of survivin isoforms, bcl-2 and p53 levels of expression by RT-PCR in different NSCLC cell lines. Our work identified a Guanine to Cytosine (G626C) point mutation in DR4 of H1299 and A549 cell lines, changing arginine to threonine (R209T). This amino acid is located in the ectodomain of DR4 which is involved in TRAIL binding. The over-expression of wt survivin in some cell lines suggest wt survivin may play a role in TRAIL resistance in SW1573 and to a lesser extent in A549, while increase of the pro-apoptotic survivin-2B may participate in the increased sensitivity of H460 to TRAIL induced apoptosis. On the other hand, results of H1299 cell line suggest that TRAIL apoptotic signaling may be different than in the other examined cell lines since high levels of anti-apoptotic survivin does not have an effect on the sensitivity of this cell line to TRAIL.

Keywords: TRAIL, bcl-2 and p53

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Apaf-1	: Apoptotic Protease Activating Factor 1
APS	: Ammonium Persulfate
ATP	: Adenosine Tri-Phosphate
Bcl-2	: B-Cell Lymphoma Predominance
BH	Bcl-2 Homology Domain
Bid	: BH3 Interacting Domain Death Agonist
BIR	: Baculovirus IAP Repeat
CARD	: Caspase Recruitment Domain
Cc	: Cubic Centimetre
cFLIP	: Cellular FLICE Inhibitory Protein
COX-2	: Cyclooxygenase
CT	: Computed Tamograph
DcR1 or DcR2	: Decoy Receptor 1 Or 2
DD	: Death Domain
DED	: Death Effector Domain
DISC	: Death Inducing Signaling Complex
DMSO	: Dimethyl Sulphoxide
DR4 or DR5	: Death Receptor 4 Or 5
DTT	: Dithiothreitol
E.M	: Electroon Microscopy
EGF	: Epidermal Growth Factor
EGFR	: Epidermal Growth Factor Receptor
EGFR	: Epidermal Growth Factor Receptor
ErbB2	:Erythroblastic Leukemia Viral [V-Erb-B] Oncogene Homologue, Avian
FADD	: Fas-Associated Death Domain
FBS	: Fetal Bovine Serum
FHIT	: Fragile Histidine Triad
FNA	: Fine Needle Aspiration
FOB	: Fiber-Optic Bronchoscopy
HIV	: Human Immunodefiency Virus
IAP	: Inhibitors Of Apoptosis
K-ras	: Kirsten rat sarcoma viral oncogene homolog
NAIP	: Neuron-Specific Members
NHL	: Non-Hodgkin's Lymphoma
NNK	:Nitrosamine-4-(Methylnitrosamino)-1-(3-Pyridil)-1-Butanone
NSCLC	: Non Small Cell Lung Cancer
P53	: Tumor protein 53
P53BSs	: P53 Binding Sites
PAHs	: Polycyclic Aromatic Hydrocarbons

PBS	: Phosphate Buffered Saline
PCD	: Programmed Cell Death
SCLC	: Small Cell Lung Carcinoma
SSCP	: Single Strand Conformation Polymorphism
TBE	: Tris Borate EDTA
TEMED	: N,N,N',N'-Tetramethylethylenediamine
TM	: Trans-Membrane
TNFR	: Tumor Necrosis Factor Receptor
TNM	: Tumor-Node-Metastasis
TRADD	: TRAIL-Associated Death Domain
TRAIL	: Tumor Necrosis Factor Related Apoptosis Inducing Ligand
WHO	: World Health Organization
Wt	: Wild Type

Introduction

Introduction

Cancer is a class of diseases characterized by uncontrolled cell growth. There are over 100 different types of cancer; each is identified by the type of cell that is initially affected.

Cancer harms the body when damaged cells divide uncontrollably to form masses of tissue called tumors (except in the case of leukemia where cancer prohibits normal blood function by abnormal cell division in the blood stream). Tumors can grow and interfere with the digestive, nervous, and circulatory systems and they can release hormones that alter body function. Tumors that stay in one region and demonstrate limited growth are generally considered to be benign. When a tumor successfully spreads to other parts of the body and grows, invading and destroying other healthy tissues, it is said to have metastasized. The result is a serious condition that is very difficult to treat. ***(Hanahan and Weinberg, 2000).***

Review of Literature

I-REVIEW OF LITERATURE

Lung cancer

1 – Incidence

Lung cancer ranks among the most commonly occurring malignancies and currently is the leading cause of cancer-related deaths worldwide ***(Peto et al., 1999)***. In the United States, lung cancer is the most common cause of cancer-related deaths in men as well as in women, with an incidence approximately 70 per 100,000 individuals ***(Jemal et al., 2004)***. Lung cancer currently accounts for one-third of all cancer-related deaths in the European Union ***(Bray et al., 1999)***.

In Egypt, lung cancer ranked the fourth most common cancer after bladder, liver and non-Hodgkin's lymphoma (NHL) with 6.1% in male and 1.3% in female with male/female ratio 4.7. Where it ranks number one in Tunisia, Algeria and Jordan. In Egypt, Non Small Cell Lung Cancer (NSCLC) including squamous cell carcinoma, adenocarcinoma accounts for about 59 % of all lung cancer patients where small and large cell carcinoma accounts for about 11% only ***(El Attar, 2005)***.

The global rise in lung cancer incidence, together with the fact that the overall 5-year survival of patients with this disease is less than 15%, underscores the magnitude of the lung cancer epidemic.

2 – Etiology

A – Smoking

The vast majority of NSCLCs are caused by cigarette smoking. In the United States approximately 80% of lung cancer deaths in men and women are directly attributable to tobacco abuse ***(Giovino, 2002).*** Cigarette smoke contains over 300 chemicals, 40 of which are known to be potent carcinogens. Of particular significance, nitrosamine 4-(methylnitrosamino)-1-(3-pyridil)-1-butanone (NNK), and polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene ***(Malkinson, 1992)***. PAH in tobacco smoke form DNA adducts, the extent of which correlates with tobacco exposure in target tissues ***(Vineis et***

al., 2004). In addition, PAHs induce mutations within the p53 tumor suppressor gene, which regulates cell-cycle progression, DNA repair, and apoptosis.

The risk of lung cancer is related to duration as well as intensity of smoking. Using data from two large case-control studies in Britain, Peto et al., (2000) observed that persistent smoking was associated with a 16-fold increase in cumulative lung cancer risk, and that this risk doubled if smoking commenced before age 15. Data from the Cancer Prevention Study II trial indicate that smoking one pack of cigarettes per day for 30 years increases risk of lung cancer–specific mortality 20- to 60-fold in men and 14- to 20-fold in women compared to risk in those who never smoked. The risk nearly doubles if consumption persists for 40 years ***(Alberg and Samet, 2003)***.

B – Genetic Predisposition

Whereas the vast majority of lung cancers are attributable to cigarette smoking, fewer than 20% of smokers develop this disease. Although these observations suggest a genetic predisposition to lung cancer, to date, the genes conferring susceptibility to this disease remain elusive. Braun *et al*. conducted a large twin cohort study and observed no genetic factors to be predictive of lung cancer ***(Braun et al., 1994)***. However, several recent studies indicate an increased risk of lung cancer among first-degree relatives of persons with this disease. Hemminki et al observed a threefold increase in lung cancer risk among siblings of patients with lung cancer ***(Hemminki et al., 2004)***. Etzel et al, observed a significant familial aggregation of lung cancer among patients with late-onset but not early-onset (before 50 years of age) lung cancer ***(Etzel et al., 2003)*.**

A number of carcinogens in tobacco smoke are potential mutagens, and several studies indicate that polymorphisms involving genes that regulate DNA repair may be associated with increased risk of lung cancer, also inflammation and reactive oxygen species contribute to the pathogenesis of lung cancer ***(Ballaz and Mulshine, 2003; Campa et al., 2003)***