سامية محمد مصطفى

شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم

سامية محمد مصطفى

شبكة المعلومات الجامعية

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

سامية محمد مصطفى

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

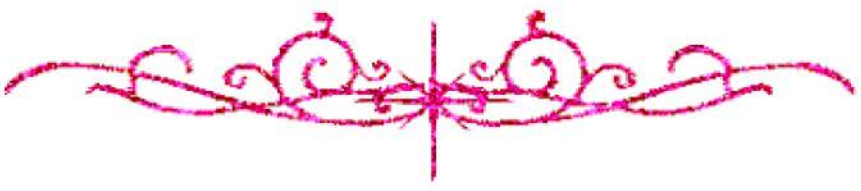
يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

سامية محمد مصطفى

شبكة المعلومات الجامعية

بعض الوثائق الأصلية تالفة



سامية محمد مصطفى

شبكة المعلومات الجامعية

بالرسالة صفحات لم ترد بالأصل

Bcl-2 AND APOPTOSIS IN COLORECTAL CANCER: A MOLECULAR AND QUANTITATIVE ANALYSIS

by

Mohamed A. Elkablawy
MBBCH, MSc.

A thesis submitted for the degree of
Doctor of Philosophy (PhD)
at the Faculty of Medicine
Queen's University of Belfast

May 2002

Quantitative Pathology Research Group
Department of Pathology
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MEMORANDUM

The accompanying dissertation is submitted in support of an application for the Degree of Doctor of Philosophy in Medicine in Queens University of Belfast.

This work has not been submitted for another Degree in this University or for an award of a Degree or Diploma of any other Institution. The help received from others has been fully acknowledged and I hereby declare that the statements in the Memorandum are true in all particulars.

Mohamed A. Elkablawy

April 2002

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Abstract

The importance of apoptosis in the pathology of disease, particularly cancer, has long been appreciated. It is well established that bcl-2 functions as a pro-survival apoptosis regulator and has implications for anti-cancer therapy. Previous studies correlating apoptosis regulatory proteins with *in situ* apoptotic rates and with clinical outcome in colorectal carcinoma (CRC) have produced conflicting results.

In this study, clinical material and cell culture models were used. The quantitative assessment of apoptotic index (AI) and mitotic index (MI) and the immunoreactivity of p53, bcl-2, $p21^{WAF1/CIP1}$ and mdm2 were examined in tumour and adjacent normal tissue samples from 30 patients with colonic and 22 with rectal adenocarcinoma. Individual features and combined profiles were correlated with clinicopathological parameters and patient survival data to assess their prognostic value. The induction of apoptosis and associated alterations in bcl-2, bax, p53, $p21^{WAF1/CIP1}$ and mdm2 protein expression and distribution were examined pre- and post-H_2O_2 treatment in a panel of five human CRC cell culture models *in vitro* (HT115, LS174T, SW480, WiDr and CACO2). An accurate quantitative assessment of bcl-2 expression was performed using flow cytometric (FCM) analysis and the relationships between bcl-2 protein expression, apoptosis and cell cycle changes were investigated in the CRC cell lines pre- and post-H_2O_2 treatment. The subcellular localisation and distribution of bcl-2 and bax proteins throughout the CRC apoptotic cells were examined using confocal laser scanning

microscopy (CLSM). Western blot (WB) analysis of purified total, nuclear and cytoplasmic protein fractions for bcl-2 and bax in all five CRC cell lines was performed and the relationship between bcl-2 expression using immunohistochemistry (IHC), FCM and WB analysis was assessed. Finally, an image processing system was developed for acquisition and processing of three-dimensional (3-D) images based on CLSM for further investigating the subcellular localisation and distribution of bcl-2 and bax proteins in CRC cells.

This study highlights the potential of using bcl-2 immunoreactivity in combination with Dukes' stage as a means of predicting favourable prognosis in CRC. A novel observation of dense nuclear localisation of chromatin-associated bcl-2 and bax protein expression in morphologically identifiable apoptotic cells and bodies in clinical and cell line materials was confirmed using WB, CLSM and 3-D reconstruction in cell line models. The staining range of bcl-2 protein family members should be extended to the nuclear compartment of human tumour cells which may have a functional role in apoptosis particularly in CRC. The FCM staining protocol was able to provide higher resolution as well as more reproducible quantitation of bcl-2 protein content in CRC cells when compared with the WB and IHC methods. Despite some future challenges, this study established a novel method of using 3-D reconstruction with CLSM datasets to provide scientifically useful results in studying intracellular protein expression. Accurate assessment of apoptosis and bcl-2 may provide valuable information on individual tumour cell dynamics for clinical purposes.

PAPERS AND PRIZES BASED ON THE WORK PRESENTED IN THIS THESIS

Published Papers

Elkablawy MA, Maxwell P, Williamson K, Anderson N and Hamilton PW: Key apoptosis and cell cycle regulatory proteins in colorectal carcinoma: relationship to tumour stage and patient survival. J Pathol 2001; 194: 436-443.

Research Prizes

The 2nd Poster prize at **XIV Congress of the International Society of Diagnostic Quantitative Pathology, (ISDQP)**, Oviedo, Spain. September, 2001.

PUBLISHED ABSTRACTS AND PRESENTATIONS BASED ON THIS WORK

Published abstracts

1. Elkablawy MA, Maxwell P, Williamson K, Anderson N and Hamilton PW: Immunohistochemical assessment of p53, $p21^{waf1/cip1}$, mdm2 and bcl-2 protein expression in colorectal carcinoma (abs).
J. Pathol 2000; 190: 10A.

2. Elkablawy MA, Maxwell P, Williamson K, Arthur K and Hamilton PW: Hydrogen peroxide-induced apoptosis associated with nuclear bcl-2 and cell cycle DNA content in colorectal cancer cells (abs).
J. Pathol 2001; 193: 25A.

3. Elkablawy MA, Maxwell P, Williamson K, Anderson N and Hamilton PW: Key apoptosis and cell cycle regulatory proteins in colorectal carcinoma: relationship to Dukes' stage and patient survival (abs).
J. Anal Cell Pathol 2001; 22: B002.

4. Elkablawy MA, Maxwell P, Williamson K, Anderson N and Hamilton PW: Evaluation of immunohistochemical scoring systems in colorectal carcinomas: association with tumour stage and patient survival (abs).
J. Anal Cell Pathol 2001; 22: X001.

5. Elkablawy MA, Maxwell P, Diamond J, Bartels PH and Hamilton PW: Nuclear texture analysis in colorectal carcinomas: association with mitosis, apoptosis, immunostaining of p53, p21, mdm2, bcl-2 and patient prognosis (abs). J. Anal Cell Pathol 2001; 22: R002.

6. Elkablawy MA, Maxwell P, Williamson K, Arthur K and Hamilton PW: Hydrogen peroxide-induced apoptosis associated with nuclear bcl-2 and cell cycle DNA content in colorectal cancer cells. (abs).
J. Anal Cell Pathol 2001; 22: H001.

7. Maxwell P, Elkablawy MA, Williamson K and Hamilton PW: Nuclear bcl-2 and bax in apoptotic colorectal cancer cells. (abs).
Cytometry 2002; Supp11: 73.

Research Presentations

Oral Presentations:

1. HYDROGEN PEROXIDE-INDUCED APOPTOSIS ASSOCIATED WITH NUCLEAR BCL-2 AND CELL CYCLE DNA CONTENT IN COLORECTAL CANCER CELLS.
2. KEY APOPTOSIS AND CELL CYCLE REGULATORY PROTEINS IN COLORECTAL CARCINOMA: RELATIONSHIP TO DUKES' STAGE AND PATIENT SURVIVAL.

3. NUCLEAR TEXTURE ANALYSIS IN COLORECTAL CARCINOMAS: ASSOCIATION WITH MITOSIS, APOPTOSIS, IMMUNOSTAINING OF P53, P21, MDM2, BCL-2 AND PATIENT PROGNOSIS.

* 7th Congress of the European Society for Analytical Cellular Pathology, Cean, France. April 2001. " X 3 presentations".

1-3. The previous 3 presentations.

4. A NOVEL INTRANUCLEAR LOCALIZATION OF BCL-2 AND BAX PROTEINS IN COLORECTAL CANCER APOPTOSIS.

5. EVALUATION OF IMMUNOHISTOCHEMICAL SCORING SYSTEMS IN COLORECTAL CARCINOMAS: ASSOCIATION WITH TUMOUR STAGE AND PATIENT SURVIVAL.

6. CHROMATIN TEXTURE SIGNATURES IN COLORECTAL CANCER LESIONS: RELATIONSHIP TO TUMOUR STAGE AND PATIENT 5 YEARS SURVIVAL.

* Cancer Research Centre (QUB), Belfast, UK.

Sebtember 1999, March 2001 and Sebtember 2001. " X 6 presentations".