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MOLECULAR GENETICS OF COLORECTAL CANCER A COMPARATIVE STUDY BETWEEN PALESTINIAN & ISRAELI PATIENTS

BY

SAMER MOHAMMAD HAMMAD OWINEH

SUPERVISOR

PROF. HISHAM DARWISH

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Committee Members	Signature
1. Dr. Hisham Darwish (Supervisor)	----------
2. Dr. Suhail Ayesh (External Examiner)	----------
3. Dr. Rasmi Abu-Helu (Internal Examiner)	----------

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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.

Signature: .....

Name: Samer Mohammad Hammad Owineh

Date: January, 5, 2004

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Abstract

Objective:

To investigate differential expression of genes including H19, Hepareanase, Ki-67, Her-2/neu, p53, p27, Cyclin D1 and B-catenin in three different populations of Colorectal Cancer patients living in the same geographical region.

Patients and Methods:

144 colorectal cancer patients' medical files (94 Palestinians and 50 Israelis (both Ashkenazi and Sephardic)) were searched for Age, gender, Grade, Stage of the disease, smoking, and 5-year survival. Samples of paraffin embedded sections and blocks for those patients were selected from pathology departments of Palestinian and Israeli hospitals.

In situ hybridization (ISH) for H19 RNA, and *Immunohistochemistry (IHC)* for Heparanase, Ki-67, Her-2/neu, p53, p27, Cyclin D1 and B-catenin were performed, and the intensity of reactions were evaluated by an authorized pathologist.

Results:

69% of Palestinian patients were between 50-72 years old. 55% were males and 45% were females. None of them was diagnosed at stage A and most of them were at B and C stages. 97% of them were at grade II. 64% of females were at B stage and 52% of males were in stage C.

60% of the Israeli patients were more than 73 years old, and were diagnosed at earlier stages and grades than Palestinian. There was no difference in H19 and Heparanase genes expression among Palestinian and Israeli patients. Heparanase expression was found 93% of all samples tested (93% for Palestinians and 96% for the Israelis).

P27 gene was expressed in 67% of the Palestinian samples compared to 33% for the Sephardic and 75% for the Ashkenazi Jews. Cyclin D1 gene was expressed in 57% of the Palestinian samples compared to 33% for the Sephardic and 25% for the Ashkenazi Jews. There were no statistical differences in the expression of p53, Her-2/neu and B-catenin among the three groups.

Conclusion: There were differences in gene expression in many genes tested on the three ethnic groups.

Abstract

الهدف: الدراسة والتحقق من تمايز تعبير مجموعة من الجينات تشمل كل من H19, Hepareanase, Ki-67, Her-2/neu, p53, p27, Cyclin D1, B-catenin في ثلاثة مجتمعات مختلفة من مرضى سرطان القولون والذين يعيشون في منطقة جغرافية واحدة.

المرضى وطرق الفحص: 144 ملف طبي لمرضى سرطان القولون تم اختيارها عشوائيا من أقسام التشريح المرضي في العديد من المستشفيات الإسرائيلية والفلسطينية، 94 منها لفلسطينيين و50 منها لإسرائيليين (يهود غربيين وشرقيين أو اليهود الاشكيناز والسفردين)، حيث تم استخراج المعلومات التالية والمتعلقة بكل مريض على حده وهي: العمر، الجنس، مرحلة المرض، تمايز الخلايا السرطانية، التدخين، النجاة لمدة خمسة سنوات بعد الإصابة، عينات من القطع السرطانية المستأصلة عبر الجراحة والمحفوظة في مادة شمعية خاصة تم أخذها وتقطيعها إلى شرائح لتتم دراستها مخبريا.
تم إجراء الفحص المسمى ب *In situ hybridization (ISH)* لجين H19 بينما تم إجراء فحص *Immunohistochemistry (IHC)* للجينات التالية Heparanase, Ki-67, Her-2/neu, p53, p27, Cyclin D1, B-catenin حيث تم تقييم تركيز التفاعلات من قبل مشرح مرضي مختص.

النتائج: 69% من المرضى الفلسطينيين تقع أعمارهم بين 50-72 سنة بينما 60% من المرضى الإسرائيليين تزيد أعمارهم عن 73 سنة، 55% من المرضى الفلسطينيين كانوا ذكورا والبقية من الإناث، لم يتم تشخيص أي مريض فلسطيني في المرحلة المبكرة A حيث تم تشخيص غالبيهم في مراحل متوسطة ومتأخرة B, C، الخلايا السرطانية كانت متوسطة التمايز عن الخلايا الطبيعية في 97% منهم، 64% من الإناث تم تشخيصهن في المرحلة B و 52% من الذكور تم تشخيصهم في المرحلة شبه المتأخرة C.
المرضى اليهود يتم تشخيصهم في مراحل مبكرة أكثر من المرضى الفلسطينيين، لم نجد أية فروقات في تعبير الجينات H19 و Heparanase بين المرضى اليهود والفلسطينيين.
لا تعبير الجيني لجين Heparanase وجد في 93% من مرضى الجانبين (92% عند الفلسطينيين و 96% عند الإسرائيليين)، كما ولوحظ تعبير لجين p27 في 67% من المرضى الفلسطينيين مقارنة مع 33% من المرضى اليهود الشرقيين و 75% من المرضى اليهود الغربيين، بينما تم ذلك في جين Cyclin D1 عند 57% من المرضى الفلسطينيين مقارنة مع 33% عند اليهود الشرقيين و 25% عند اليهود الغربيين.
لم نجد أية فروقات ذات دالة إحصائية في تعبير الجينات Her-2/neu, p53, B-catenin بين الثلاثة مجتمعات المختلفة.

***الخلاصة:** يوجد العديد من الاختلافات بين مرضى سرطان القولون الفلسطينيين واليهود ومن ضمنها الاختلاف الجيني.*

INTRODUCTION

1.1. Background of Colorectal Cancer

The word Cancer is a popular term for a Malignant Neoplasm. Cancers arising in epithelial cells are called Carcinomas. Cancers arising in the Mesenchymal tissues are called Sarcomas. Carcinomas are further divided into Adeno-carcinomas when they have a glandular growth pattern or Squamous Cell Carcinomas when they produce recognizable squamous cells. Sometimes the cancer is composed of undifferentiated cells and designated as a poorly differentiated or undifferentiated malignant tumor or undifferentiated carcinoma or sarcoma (Cotran *et al.,* 1999).

After a quarter century of rapid advances, cancer research has generated a rich and complex body of knowledge, revealing cancer to be a disease involving dynamic changes in the genome. Where mutations produce oncogenes with dominant gain of function and tumor suppressor genes with recessive loss of function in human and animal cancer cells (Hanahan and Weinberg 2000), this makes the cell lose its normal control and start to divide in an uncontrolled manner (Coleman *et al.,* 1997). The cell will also look different in terms of shape, size and its internal components. The transformed properties are transferred upon all of the daughter cells. Cancer cells have properties of endless replication, loss of contact inhibition, invasiveness and the ability to metastasize (Morson, 1988).

Colorectal cancer is the commonest cause of death due to malignancy in nonsmokers in western countries and consumes considerable health care resources (Esteve *et al.,* 1993). There has been marginal improvement in survival from the disease in the past 50 years and there is a need to improve understanding of the fundamental mechanisms of colorectal carcinogenesis to facilitate development of

new approaches aimed at arresting and even preventing the malignant process (Coleman *et al.,* 1993; Chu *et al.,* 1994).

Clinically cancer begins with signs and symptoms of disease in the patient followed by direct visualization by imaging and biopsy, while pathologically it is the gross and microscopic morphological changes, and at the molecular level it is the presence of specific and sequential clonal genetic alterations (Cairns *et al.,* 1999). Traditionally, carcinogenesis has been thought of consisting of three major stages initiation, promotion and progression. Initiation involves the normal genome undergoing an irreversible genetic change to an altered genome; promotion is the shift from the normal process of cell differentiation to growth, and then progression to local tumor and metastases (Vogelstein *et al.,* 1988).

1.1.1. Anatomy and Histology of the Colon

The Colon & Rectum or Large Bowel is a storage and absorptive organ. It starts with the Cecum where the Appendix is attached to the lower part of the Cecum. The next part is the Ascending Colon, which runs up to the Hepatic Flexure situated just under the Liver. The Transverse Colon runs transversely across the abdomen to the Splenic Flexure lying just below the Spleen. Colon then runs downwards and is called the Descending Colon down to the left lower abdominal quadrant where it makes an S-shaped loop called the Sigmoid Colon, which runs down into the Pelvis and is called the Rectum. Rectum is about 13 cm long and goes over into the Anal Canal, which ends with the Anus (Silverstein *et al.,* 1995), these features are clearly illustrated in Figure1.1.

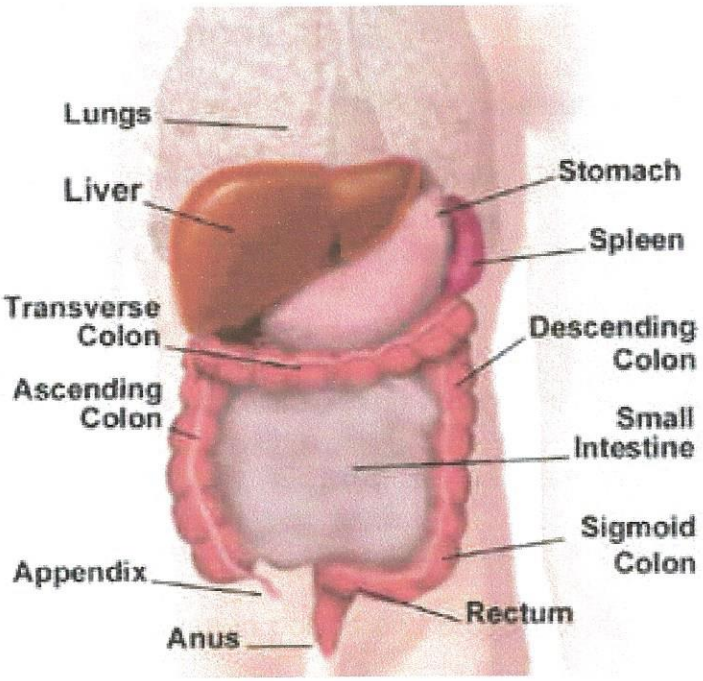

Figure1.1: the human large intestine including the main parts of the colorectal canal; starting with the Appendix, Ascending Colon, Hepatic Flexure, Transverse Colon, Splenic Flexure, Descending Colon, S-shaped Sigmoid Colon, Rectum, and the Anal Canal, which ends with the Anus (Goldinger, 2000).

The colorectal wall is made up of four layers - the Mucosa, the Submucosa, the Muscularis Propria and the Serosa. The Mucosa is closest to the lumen and consists of, starting from the lumen - the Epithelium (simple columnar), Lamina Propria and Muscularis Mucosae. Followed by the Submucosa, and the Muscularis propria. The Serosa is the external coat consisting of a layer of Mesothelial cells resting on loose connective tissue outside the Muscular layer (Morson, 1988), these features are clearly illustrated in Figure2.1

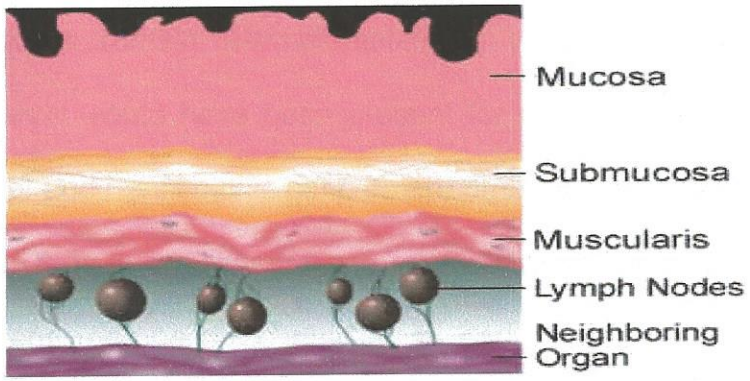

Figure 2.1: The colorectal wall including four layers - the Mucosa, Submucosa, Muscularis Propria and Serosa, see also lymph nodes and neighboring organ (Goldinger, 2000).

1.1.2. Colorectal Cancer

Colorectal Cancer arises in the epithelial cells outlining the lumen of the Colon and Rectum (a Simple Glandular Columnar Epithelium). The cancer is thus called a Colorectal Adenocarcinoma. There are other forms of Colorectal Cancer like Squamous Cell Carcinoma, Sarcoma, Lymphoma and Carcinoid Tumors but they are very rare and altogether constitute less than 2% of the cases in humans, consequently therefor, virtually, 98% of all cancers in the large intestine are adenocarcinomas. They represent one of the prime challenges to the medical profession because they arise in polyps and produce symptoms relatively early and at a stage generally curable by resection (Cotran *et al.*, 1999).

Several lines of evidence indicate that tumorigenesis in humans is a multistep process and these steps reflect genetic alterations that drive the progressive transformation of normal human cells into highly malignant derivatives (Coleman *et al.*, 1997). Pathological analyses reveal lesions that appear to represent the intermediate steps between pre-malignant stages and highly invasive cancers (Hanahan and Weinberg 2000).

1.1.3. Symptoms

Colorectal cancers remain asymptomatic for years; symptoms develop insidiously and frequently would have been present for months, sometimes years, before diagnosis. Cecal and right colonic cancers are often called to clinical attention by the appearance of fatigue, weakness, and iron deficiency anemia. The bulky lesions bleed readily and may be discovered at an early stage. Left sided lesions come to attention by producing occult bleeding, changes in the bowel habits, or crampy left-lower-quadrant discomfort. In theory, the chance for early discovery and successful removal should be greater with lesions on the left side because these

patients usually have prominent disturbances in bowel function, such as melena, diarrhea, and constipation (Fong *et al.,* 1995). Cancers of the rectum and sigmoid, however, tend to be more infiltrative at the time of diagnosis than proximal lesion and therefore have a somewhat poorer prognosis. All colorectal tumors spread by direct extension into adjacent structures and by metastasis through the lymphatic and blood vessels. In order of preference, the favored sites of metastatic spread are the regional lymph nodes, liver, lungs, and bones, followed by other sites, including the serosal membrane of peritoneal cavity and brain (Hoebler, 1997).

1.1.4. Diagnosis

Abdominal Examination, a Digital Rectal Exam and a Rigid Sigmoidoscopy. Physicians usually order examinations like Blood Samples, FOBT (Fecal Occult Blood Test), Colonoscopy, and Barium Enema. Colonoscopy is a very effective way to visualize the entire Colon and Rectum. During Colonoscopy, the bowel can be examined, polyps can be removed, tumors that require surgery can be found and biopsies can be obtained for further pathological examinations. (Cohen *et al.,* 1993)

If Colorectal Cancer is confirmed a Biopsy is examined to reveal the histology, blood tests and Chest X-ray are ordered. An Ultrasound of the liver may also be done. If the cancer is large, especially if it's a rectal cancer, Magnetic Resonance Imaging, Computer Tomography and or Transrectal Ultrasound may be required in order to see if the tumor invades adjacent structures (Kemeny *et al.,* 1993; Parker *et al.,* 1996).

1.1.5. Epidemiology

The Global population is now estimated to reach approximately 6,1 billion people. *Every year,* 133 million people are born and 52 million people die. The

median Average life expectancy is 65 years. Where 12% of the population (6,3 million) *die from* Cancers every year, which is the 3rd most common cause of death after Infectious & Parasitic diseases from which 33% (17,3 million) die, and Diseases of the Circulatory System from which 29% (15,3 million) dies every year. There are big differences in what people die from in the Developed and Developing world. Cancers account for 21% of all deaths in the Developed world, and only 9% of all deaths in the under developed world (Goldinger, 2000).

Worldwide about 8 million people *get cancers every year.* About 876,000 people get Colorectal Cancer every year, which makes it the 3rd most common type of cancer to strike people worldwide every year after (1) Lung and (2) Stomach Cancers. Worldwide about 6.3 million people die from cancer every year. Of which 525,000 people die from Colorectal Cancer yearly, which makes it the 3rd common cause of cancer death worldwide after Lung and Stomach Cancers (Ilyas *et al.,* 1996).

Concerning the incidence rate approximately 15 people out of every 100,000 people come down with Colorectal Cancer every year, that differs considerably between countries. It is highest in the Industrialized Countries where it reaches about 50 and lowest in Asia and Africa with Incidence Rates down to 1 (Cohen & Winawer, 1995; Landis et *al.,* 1998). The Incidence Rate for Colorectal Cancer is extremely low in childhood but increases with age (Wingo *et al.,* 1995).

1.1.6. Colorectal Polyps

Polyps of the colorectal mucosa are extraordinarily common in older adult population. Several concepts pertaining to terminology must be emphasized:

- A polyp is a tumorous mass that protrudes into the lumen of the gut.