

EXPRESSION OF CATHEPSIN D IN
COLORECTAL CARCINOMA
Immunohistochemical and
Histopathological Study

Thesis

**Submitted For Partial Fulfillment of M.D Degree in
Pathology**

By

Rasha Ahmed Khairy Abd El Gawad
M.B.B.Ch, M.Sc., Cairo University

Supervisors

Prof. Dr. Badawia Bayoumi Ibrahim
Professor of Pathology
Faculty of Medicine, Cairo University

Dr. Dina Omar Helmy
Assistant Professor of Pathology
Faculty of Medicine, Cairo University

Dr. Mostafa Mahmoud Khodeir
Assistant Professor of Pathology
Faculty of Medicine, Cairo University

Faculty of Medicine
Cairo University

2012

ABSTRACT

Colorectal cancer is one of the leading causes of cancer death in both developed and developing nations. It is the third most common type of cancer and the second leading cause of cancer death in the United States. The current study included fifty cases of colorectal carcinomas studied histologically, and immunohistochemically for Cathepsin D expression. Cathepsin D expression was observed in 90% of cases in tumor cells and in 92 % of cases in peritumoral stromal cells of colorectal carcinomas. All cases of mucinous and signet ring cell carcinoma and 87.2% of adenocarcinomas showed Cathepsin D positive immunostaining. Statistically significant relationship was detected between Cathepsin D expression in cancer cells and histologic grade but not with lymph node metastasis or stage, however, statistically significant relationship was detected between Cathepsin D expression in peritumoral stromal cells and depth of tumor invasion and , lymph node metastasis. In addition, statistically significant relationship was detected between Cathepsin D immunostaining pattern in tumour cells and histologic grade as in more poorly differentiated tumors, there was a diffuse cytoplasmic staining while in better differentiated tumors there was apical staining pattern reflecting loss of cellular polarization in cancer cells. Therefore, it is suggested that Cathepsin D may be used as a prognostic marker in detection of invasiveness and metastatic potential in colorectal carcinomas and it will be necessary to carry out similar studies on a larger sample size and look for correlations between Cathepsin D expression and survival rate.

Key words: Colorectal carcinoma, adenocarcinoma, mucinous, signet ring, Cathepsin D.

Acknowledgement

First and foremost **"Thanks to GOD"**, the most merciful and kind.

I am honored to have **Prof. Dr. Badawia Bayoumi Ibrahim** Professor of Pathology, Faculty of Medicine, Cairo University, as a supervisor of this work. I am deeply grateful and most appreciative to her great efforts, kind guidance and valuable advices that encouraged and helped me to finish this work.

My profound gratitude goes to **Dr. Dina Omar Helmy** Assistant Professor of Pathology, Faculty of Medicine, Cairo University for her meticulous supervision, constructive criticism and precious remarks throughout the course of this study.

Special thanks are owed to **Dr. Mostafa Mahmoud Khodeir**, Assistant Professor of Pathology, Faculty of medicine, Cairo University, who saved no time and effort in helping me, his constant support, continuous encouragement and constructive comments allowed me to accomplish this work.

Finally, I would like to thank my precious family for their patience, love, motivation and support throughout this work.

Rasha Ahmed Khairy

List of Abbreviations

ACF	: Aberrant crypt foci
AJCC	: American Joint committee on Cancer
APC	: Adenomatous polyposis coli
BAX	: Bcl 2 associated X protein
BCL 2	: B cell lymphoma 2
BMI	: Body mass index
CAP	: Collage of American Pathologist
CD	: Crohn's disease
CEA	: Carcinoembryonic Antigen
CK	: Cytokeratin
CRC	: Colorectal Carcinoma
CRM	: Circumferential margin
DAB	: Diaminodenzidine
DCC	: Deleted in colon cancer
DNA	: Deoxy ribonucleic acid
DPC 4	: Tumor suppressor gene
EC	: Enterochromaffin
ECM	: Extracellular matrix
EGF	: Epidermal growth factor
ELISA	: Enzyme linked immunosorbent assay
FAP	: Familial adenomatous polyposis
GH	: Growth hormone
GLUT 1	: Glucose transporter 1
HCG	: Human Chorionic gonadotropins
H & E	: Hematoxylin and Eosin
HGD	: High-grade dysplasia
HIV	: Human immunodeficiency virus
HMLH 1	: Mismatch repair genes
HNPCC	: Hereditary Non Polyposis Colorectal Carcinoma
HP	: Hyperplastic polyps
ICAM 1	: Intercellular Adhesion Molecule 1

IEN	: Intraepithelial neoplasia
IGF 1	: Insulin like growth factor 1
Ig A	: Immunoglobulin A
K RAS	: Oncogene
LGD	: Low grade dysplasia
LN	: Lymph node
M6Pr	: Mannose 6-phosphate receptor
MSI	: Microsatellite instability
MUC	: Mucin
NEC	: Neuroendocrine carcinoma
NET	: Neuroendocrine tumour
P 53	: Tumour suppressor gene
PJS	: Peutz-Jeghers syndrome
PP	: Pancreatic Polypeptide
P value	: Probability value
PYY	: Peptide Tyrosine Tyrosine
RERs	: Replication errors
SCC	: Squamous cell carcinoma
SMAD 4	: Tumour suppressor gene
SPSS	: Statistical Product for Services Solution
SSA	: Sessile serrated adenoma
TSA	: Traditional serrated adenoma
TNF	: Tumour necrosis factor
UC	: Ulcerative colitis
UICC	: Union International Contre Le Cancer
US	: United States
WCRF/AICR	: World Cancer Research Fund/American Institute for Cancer Research
WHO	: World Health Organization

List of Tables

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
1.	Regional lymph node groups in anatomic subsites of the colorectum	7
2.	WHO histological classification of tumours of the colon and rectum	10
3.	Vienna Classification of Gastrointestinal Neoplasia	21
4.	Criteria for histologic grading of colorectal adenocarcinoma	34
5.	TNM staging classification for cancers of the colon and rectum, AJCC (2010)	50
6.	Stage grouping	50
7.	Modified Dukes' staging of colorectal cancer	68
8.	Modified Dukes' staging of studied cases	77
9.	The relationship between histologic grade and LN metastasis	80
10.	The relationship between histologic grade and Modified Dukes' stage	81
11.	The relationship between histologic grade and depth of tumor invasion	82
12.	The relationship between histologic type and LN metastasis	82
13.	The relationship between tumor site and gross pattern	84
14.	The relationship between scoring of Cathepsin D expression in tumor cells and age	84
15.	The relationship between scoring of Cathepsin D expression in tumor cells and sex	85
16.	The relationship between scoring of Cathepsin D expression in tumor cells and tumor site.	86
17.	The relationship between scoring of Cathepsin D expression in tumor cells and tumor site.	86
18.	The relationship between scoring of Cathepsin D expression in tumor cells and histologic grades.	87
19.	The relationship between pattern of Cathepsin D immunoexpression in tumor cells and histologic grades	88
20.	The relationship between scoring of Cathepsin D expression in tumor cells and LN metastasis.	89

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
21.	The relationship between Cathepsin D immunostaining pattern in tumor cells and LN metastasis.	89
22.	The relationship between scoring, of Cathepsin D expression in tumor cells and modified Dukes' stage.	91
23.	The relationship between Cathepsin D immunostaining pattern in tumor cells and modified Dukes' stage.	91
24.	The relationship between scoring of Cathepsin D expression in tumor cells and depth of tumor invasion.	92
25.	The relationship between Cathepsin D immunostaining pattern in tumor cells and depth of tumor invasion.	93
26.	The relationship between Cathepsin D expression in stromal cells and histologic types.	93
27.	The relationship between Cathepsin D expression in stromal cells and LN metastasis.	94
28.	The relationship between Cathepsin D expression in stromal cells and modified Dukes' stage.	95
29.	The relationship between Cathepsin D expression in stromal cells and depth of tumor invasion.	96

List of Graphs

<i>Graph No.</i>	<i>Graph Title</i>	<i>Page No.</i>
1.	Age distribution in the studied cases.	72
2.	Sex distribution in the studied cases	73
3.	Distribution of cases according to their site	73
4.	Distribution of cases according to their gross pattern	74
5.	Distribution of cases according to Histologic type	74
6.	Distribution of cases according to Histologic grade	75
7.	Distribution of cases according to depth of tumor invasion	75
8.	Distribution of lymph node metastasis in studied cases	76
9.	Distribution of cases according to distant metastasis presentation	76
10.	Distribution of cases according to modified Dukes' stage	77
11.	Scoring of Cathepsin D expression in tumor cells of studied cases	78
12.	Cathepsin D staining pattern in tumor cells of studied cases	78
13.	Cathepsin D expression in stromal cells of studied cases	79
14.	The relationship between histologic grade and LN metastasis	80
15.	The relationship between histologic grade and Modified Dukes' stage	81
16.	The relationship between histologic type and LN metastasis	83
17.	The relationship between scoring of Cathepsin D expression in tumor cells and sex	85
18.	The relationship between pattern of Cathepsin D immunoexpression in tumor cells and histologic grades	88
19.	The relationship between Cathepsin D immunostaining pattern in tumor cells and LN metastasis.	90
20.	The relationship between Cathepsin D expression in stromal cells and LN metastasis	94
21.	The relationship between Cathepsin D expression in stromal cells and modified Dukes' stage.	95
22.	The relationship between Cathepsin D expression in stromal cells and depth of tumor invasion.	96

List of Figures

<i>Figure No.</i>	<i>Figure Title</i>	<i>Page No.</i>
1.	Anatomy of large intestine	5
2.	Dukes staging of CRC	49
3.	Diagrammatical representation of a resected rectum.	54
4.	Diagrammatic illustration of rectal tumours in relation to the peritoneal reflection	54
5.	Measuring extramural spread and clearance of tumour from the non-peritonealised margin	54
6.	Well differentiated colonic adenocarcinoma showing mildly irregular glands surrounded by desmoplastic stroma. (H&E x100)	97
7.	Moderately differentiated colonic adenocarcinoma formed of irregular and branching glands with surrounding inflamed desmoplastic stroma. (H&E x100)	97
8.	Moderately differentiated colonic adenocarcinoma formed of branching glands infiltrating the muscle layer. (H&E x100)	98
9.	Moderately differentiated colonic adenocarcinoma formed of irregular glands exhibiting nuclear stratification and moderate atypia. (H&Ex400)	98
10.	Poorly differentiated colonic adenocarcinoma formed of infiltrating groups and cords surrounded by inflamed desmoplastic stroma. (H&E x200)	99
11.	Poorly differentiated colonic adenocarcinoma showing pericolic fat infiltration by diffuse sheets formed of highly pleomorphic tumor cells exhibiting high degree of atypia and frequent prominent nucleoli (H&E x 400)	99
12.	Moderately differentiated colonic adenocarcinoma showing irregular glands infiltrating pericolic fat. (H&E x 400)	100
13.	Complex glandular architecture creating irregular cribriform pattern in moderately differentiated colonic adenocarcinoma (H&E x 400)	100
14.	Colonic mucinous adenocarcinoma showing fused glands with cribriform pattern floating in lakes of extracellular mucin. (H&Ex100)	101

<i>Figure No.</i>	<i>Figure Title</i>	<i>Page No.</i>
15.	Colonic mucinous adenocarcinoma showing irregular and fused glands with surrounding lakes of extracellular mucin (H&E x200)	101
16.	Diffuse muscle infiltration by signet ring cells in colonic signet ring cell carcinoma. (H&E x 400)	102
17.	Strong diffuse cytoplasmic Cathepsin D expression in well differentiated colonic adenocarcinoma (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x100)	102
18.	Positive diffuse cytoplasmic Cathepsin D expression in irregular glands in moderately differentiated colonic adenocarcinoma and in peritumoral stromal cells. (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x100)	103
19.	Diffuse cytoplasmic Cathepsin D immunostaining in solid islands with focal cribriform pattern in poorly differentiated colonic adenocarcinoma (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x100)	103
20.	Strong diffuse cytoplasmic Cathepsin D expression in malignant cords infiltrating the pericolic fat in poorly differentiated colonic adenocarcinoma (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x200)	104
21.	Positive diffuse cytoplasmic Cathepsin D expression in poorly differentiated colonic adenocarcinoma and peritumoral stromal cells and negative adjacent normal colonic mucosa (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x100)	104
22.	Moderately differentiated colonic adenocarcinoma showing glands exhibiting supranuclear Cathepsin D immunostaining pattern and positive stromal cells (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x200)	105
23.	Glands showing supranuclear Cathepsin D immunostaining pattern in moderately differentiated colonic adenocarcinoma (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x100)	105
24.	Glands showing supranuclear Cathepsin D immunostaining pattern in well differentiated colonic adenocarcinoma (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x400)	106

<i>Figure No.</i>	<i>Figure Title</i>	<i>Page No.</i>
25.	Diffuse cytoplasmic Cathepsin D immunostaining in tumor cells surrounding pools of mucin in colonic mucinous carcinoma (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x100)	106
26.	Cathepsin D positive immunostaining in tumor and stromal cells in mucinous adenocarcinoma (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x 100)	107
27.	Signet ring cells showing positive cytoplasmic immunostaining of Cathepsin D in colonic signet ring cell carcinoma infiltrating the muscle (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x 400)	107
28.	Signet ring cells showing positive cytoplasmic immunostaining of Cathepsin D in colonic signet ring cell carcinoma (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x 200)	108
29.	Positive Cathepsin D expression in peritumoral stromal cells and negative in tumor cells in poorly differentiated colonic adenocarcinoma (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x100)	108
30.	Moderately differentiated colonic adenocarcinoma showing irregular glands exhibiting diffuse cytoplasmic Cathepsin D immunostaining and positive stromal cells (Cathepsin D antibody with DAB chromogen and hematoxylin counterstain x100)	109

CONTENTS

	<i>Page</i>
INTRODUCTION	1
AIM OF WORK	3
REVIEW OF LITERATURE:	
▪ Normal Large Intestine	4
▪ Classification of Tumors of Large Intestine	10
▪ Etiology of Colorectal Cancer.....	11
○ Epidemiology	11
○ Risk Factors	13
○ Precursor Lesions	19
○ Pathogenesis	25
▪ Clinical Features of Colorectal Cancer	30
▪ Gross Features of Colorectal Carcinoma.....	31
▪ Histopathological Features of Colorectal Carcinoma.....	32
▪ Spread of Colorectal Cancer	45
▪ Prognostic Factors of Colorectal Cancer.....	48
▪ Cathepsin D	60
MATERIALS AND METHODS	67
RESULTS	72
DISCUSSION	110
SUMMARY	121
CONCLUSION AND RECOMMENDATIONS	123
REFERENCES	125
ARABIC SUMMARY	

INTRODUCTION

Colorectal cancer is the third most common type of cancer and the second leading cause of cancer death in the United States (**Calonge et al., 2008**). It ranks fourth in frequency in men and third in women all over the world (**Parkin et al., 2005**).

In Egypt, the Cancer Pathology Registry of National Cancer Institute of Cairo University showed that during the years 2003-2004, colorectal cancer occupied the first rank among digestive system's malignancies (15.78%) and the fifth rank among all total cancers (4.34%) (**Mokhtar et al., 2007**).

The prognosis of patients with colorectal carcinoma is mainly dependant on the staging systems which represent a combination of the criteria of local extent and lymph node involvement, whether ones uses the original scheme proposed by Dukes or any of the modifications that have been subsequently advanced (American Joint Committee category IIA) (**Rosai J, 2004**).

Selection of the most beneficial treatment regimens in colorectal cancer remains a challenge and is hindered by a lack of predictive and prognostic markers. In recent years, research on a global scale has attempted to define subsets of prognostic markers to determine the aggressiveness of the disease and the like hood of recurrence after surgery (**Wilson et al., 2007**).

The metastatic process in cancer depends on the invasion of the tumor cells to the surrounding matrix and penetration of the basement

membrane to reach the systemic circulation. These steps of degradation and membrane passage are used to be basically controlled by tumor associated proteolytic enzymes. These include endopeptidases, matrix metalloproteinase and cathepsins (**Tetu et al., 2001**).

Cathepsin D is known to be a lysosomal acid protease that is mainly included in intracellular protein catabolism and is inducible by estrogens. Therefore, many investigators have studied intensively the role of Cathepsin D in breast carcinomas. These studies have suggested that the expression of Cathepsin D is correlated with the invasion and metastasis of breast carcinomas, but several studies done to investigate the role of Cathepsin D expression in predicting prognosis or invasive potential in colorectal carcinoma revealed conflicting results (**Yilmaz et al., 2003**).

Several studies have reported a wide range of Cathepsin D and their antigen expressions patterns in colorectal tumours with the development of the disease stage, suggesting the use of Cathepsin D as a prognostic tumor marker in colorectal cancer (**Sebzda et al., 2005**).

AIM OF THE WORK

The current work consists of histopathological and immunohistochemical studies on colorectal carcinoma (CRC) designed to assess and investigate:

1. The expression of Cathepsin D in the different histologic types of colorectal carcinoma.
2. Assess the role of Cathepsin D in predicting progression, invasive and metastatic potential of colorectal carcinoma.