EVALUATION OF THE REGULATORY ROLE OF TGF-β SIGNALING IN THE MALIGNANT TRANSFORMATION OF INFLAMMATORY BOWEL DISORDERS IN EGYPTIAN PATIENTS

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Ihab Abdel Moneam

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AKT	Alpha serine/threonine protein kinase
AOSD	Adult-onset Still's disease
CD	Crohn's disease
CDKN1A	Cyclin Dependent Kinase Inhibitor 1A
CRC	Colorectal cancer
CXCR	Chemokine receptor
DGCR8	DiGeorge Syndrome Critical region 8
EGFR	Epidermal growth factor receptor
eIF4F	Eukaryotic initiation factor 4F
EMT	Epithelial mesenchymal transition
ERK	Extracellular signal-regulated kinase
FAP	Familial adenomatous polyposis
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GATA3	GATA binding protein 3
IBD	Inflammatory bowel disease
IFNγ	Interferon gamma
LNM	Lymph node metastasis
MEK	Mitogen-activated protein kinase kinase
MMP	Matrix metalloproteinase
mTOR	Mammalian target of rapamycin
NF-κB	Nuclear factor-kappaB
PI3K	Phosphoinositide-3-kinase
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PMNs	Polymorphonuclear neutrophils
PTEN	Phosphatase and tensin homolog
Ras-MAPK	Mitogen-activated protein kinase
RORγ	RAR-related orphan receptor gamma
SMURF1/2	Smad ubiquitylation regulatory factor 1/2
STAT3	Signal transducer and activator of transcription 3
Tbet	T-box transcription factor
TGF-β	Transforming growth factor beta
Th17	T helper 17 cell
TNM	Tumor, node, metastasis
Treg	$CD4^+$ or $CD25^+$ regulatory T-cell
TβR	Transforming growth factor beta receptor
UC	Ulcerative colitis
VEGF	Vascular endothelial growth factor

INTRODUCTION

Cancers can be caused by manners of changes in gene expression. Genes that induce cancerogensis can be largely called as oncogenes, which are activated by alterations; and tumor suppressor genes, which are repressed during cancerogenesis. Oncogenes can encode growth factors or their receptors, signaling molecules, regulators of the cell cycle, and other factors that control cell proliferation and survival. Their oncogenicity can be mediated by mutations that lead to overexpression of gene products, amplifications that alter copy number, alterations or rearrangements that affect promoter function, or modified interactions with regulators of transcription or epigenetic modification. Tumor suppressors repress growth and proliferation, passage through the cell cycle, motility, invasion, or other functions related to stable differentiation. Colorectal cancers (CRCs) grow gradually over a long period of time through the sequential accumulation of genetic mutations that lead to the excrescent control mechanisms built into each cell. **(Wood et al., 2007)**

Colorectal cancer

Colorectal cancer (CRC) is one of the most common malignant tumor, and the third leading cause of cancer related death in the world **(Siegel et al., 2017)**. Colorectal cancer (CRC) is considered a main cause of both morbidity and mortality. Its occurance is always increasing in the developing countries **(Agyemang-Yeboah et al., 2017)**. In Egypt, CRC represents 6.5% of all cancers **(El-Bolkainy et al., 2005)**. Further, the National Cancer Institute registry, Cairo University for the years (2002) - (2003) cleared CRC was among the most common cancers registered (6th); it was 4.2% in males and

3.8% in females **(Ibrahim et al., 2014)**. Also, CRC has been detected in about 13% of the patients undergoing colonoscopy in Egypt, and the incidence is increasing annually **(Gado et al., 2014)**.

Colorectal cancer is caused by the abnormal growth of epithelial cells which form the lining of the colon or rectum. These small growths (known as polyps) are often benign, although some may develop into malignant. It is detected that up to two thirds of colorectal polyps are pre-malignant and represents a risk of colorectal cancer **(Levin et al., 2008)**.

There are several risk factors that may increase the chance of an individual developing colorectal cancer.

Risk factors:

- **Family history**: A person's risk doubles if a direct relative has previously had the disease. There is an even greater risk if more than one relative has had colorectal cancer **(Shaukat et al., 2017)**.
- **Genetics**: Individuals with inherited disorders such as familial adenomatous polyposis (FAP), where an individual is prone to polyp formation, have a higher risk of developing colorectal cancer **(Shaukat et al., 2017)**.
- **Colorectal polyps or inflammatory bowel diseases**: A history of polyps or inflammatory bowel disease, where the bowel is inflamed for many years, increases the risk of colorectal cancer **(Gupta et al., 2007)**.
- **Age**: Although a person can develop colorectal cancer at any age, the risk increases greatly with age. Over 90% of colorectal cases are diagnosed in patients over the age of 50 **(Raskov et al., 2014)**.

- **Lifestyle**: A sedentary lifestyle is associated with a higher risk of colorectal cancer. Studies have also linked obesity, lack of exercise, smoking and excessive alcohol consumption to a greater risk of colorectal cancer **(Pan et al., 2018)**.

Early diagnosis of colorectal cancer has the potential to improve survival rates; however early symptoms (such as abdominal pain) may be confused with other diseases, **(John et al., 2011)** meaning many patients have advanced disease when diagnosed **(Henley et al., 2010).** Almost 85% of patients referred to hospital have one or more of the following high-risk symptoms **(Flashman et al., 2004)**:

- Rectal bleeding
- A mass in the abdomen or rectum
- Change in bowel habit
- Perianal symptoms, such as abscesses or lesions

As the cancer becomes more advanced, other symptoms can develop. For example, excessive bleeding from the colon can cause anaemia, which leaves the patient feeling breathless and tired. If the cancer begins to obstruct the colon, further symptoms include bloating, constipation and vomiting **(Calva et al., 2009)**. high-risk symptoms to their doctor they will be given a physical examination. If this raises any concerns, a number of additional tests may be performed:

- Colonoscopy – the entire length of the colon is viewed using a colonoscope.
- Sigmoidoscopy – a small tube (sigmoidoscope) is used to view the lower colon.

- Double contrast barium enema – x-rays of the colon and rectum. Barium lines the colon allowing an outline to be viewed in an x-ray.

A biopsy, where sample tissue is removed during a colonoscopy or sigmoidoscopy, is required to confirm the diagnosis of colorectal cancer and determine how advanced the disease is (staging) **(Cress et al., 2009).**

Staging of colorectal cancer determines how advanced the cancer is and whether it has spread to other parts of the body. It helps to identify the most appropriate treatment options for the patient. Staging in colorectal cancer can be confirmed by **(Saunders et al., 2002)**:

- Blood tests to look for tumour markers
- Biopsies, analysing tissue samples taken during a colonoscopy or sigmoidoscopy
- Imaging tests (CT scans, chest x-rays, ultrasound, MRI scans)
- Surgery.

The most common staging for colorectal cancer is defined by the tumour, node, metastasis (TNM) staging system, which classes a patient into stages I-IV according to the level of invasion or spread of the tumour to other organs (metastasis). Using the TNM staging, the progression of the original primary tumour is denoted by the letter T (tumour); N (node) indicates whether the tumour has spread to lymph nodes; M (metastasis) represents whether the tumour has metastasised to distant organs in the body, most commonly the liver or lungs. T, N and M are followed by numbers giving further information on the stage of the disease: increasing numbers signify later stages **(AJCC, 2010; Greene and Sobin, 2008).**

Early-stage' disease (stage I and II) describes a tumour that has not yet spread to the lymph nodes or other distant areas in the body. With early-stage disease there is the chance of cure if the tumour can be successfully surgically removed. When cancer spreads from the original site, affecting the lymph nodes (stage III) or other parts of the body (stage IV), treatment becomes more difficult (Table 1, figure 1) **(AJCC, 2010)**.

Table 1: The stages of colorectal cancer (TNM).

Stage	Classification
Stage I	The tumour is localised to the lining of the colon. T1–T2, N0, M0
Stage II	The tumour grows into the outer lining of the colon or surrounding tissue. T3–T4, N0, M0
Stage III	The cancer has metastasised to the lymph nodes. Any T, N1–N2, M0
Stage IV	The cancer has metastasised to distant organs in the body. Any T, Any N, M1

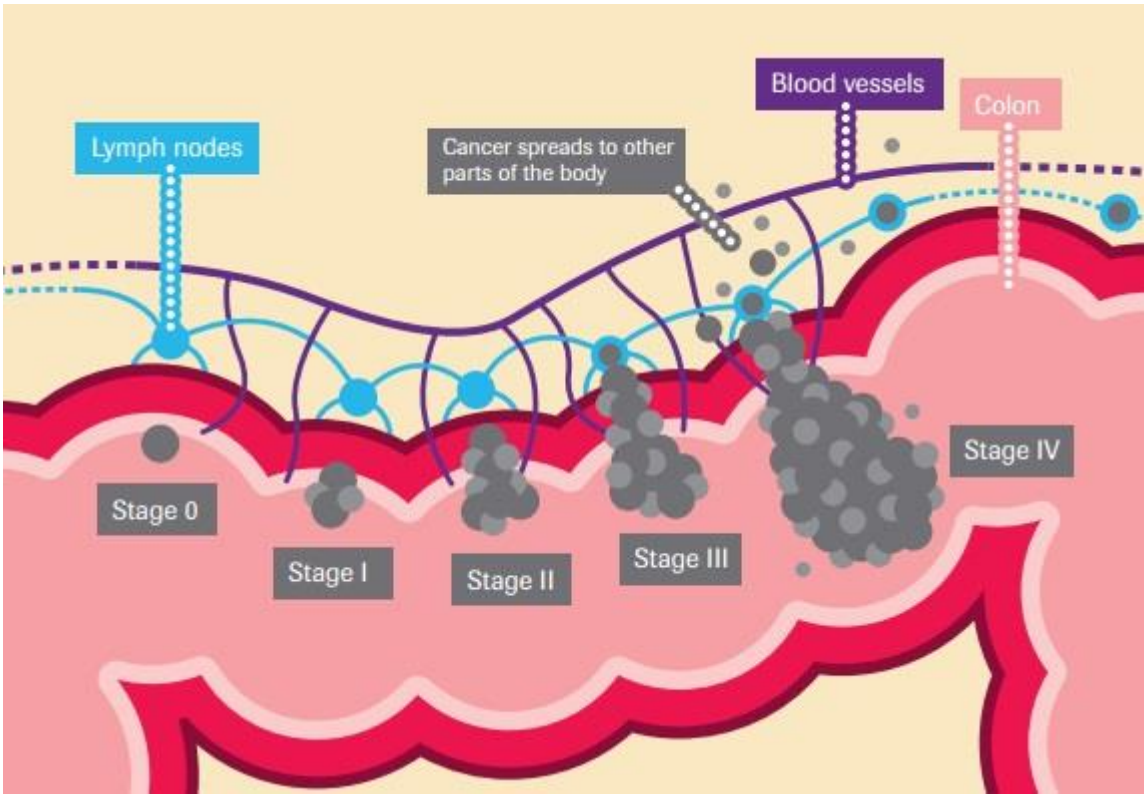

Figure 1: Stages of colorectal cancer (AJCC, 2010).

Accumulating evidence suggests that inflammation plays a crucial role in colorectal carcinogenesis and progression. The susceptibility to colorectal cancer (CRC) is strongly increased in patients with inflammatory bowel disease (IBD) or ulcerative colitis and Crohn's disease **(Rubin et al., 2012)**

Inflammatory bowel disease (IBD):

Inflammatory bowel disease (IBD) describes conditions with idiopathic, chronic, relapsing and remitting inflammation of the gastrointestinal tract. Crohn's disease (CD) and ulcerative colitis (UC) are the two most common types of IBD. UC is limited to the colon (large intestine); CD can involve any part of the gastrointestinal tract from the mouth to the anus, although it most commonly affects the small intestine and/or the colon. About 5-15% of patients with IBD affecting the colon have features of both conditions

(Mowat et al., 2011). IBD leads to significant morbidity and to impaired quality of life, generally without affecting mortality **(Andrews et al., 2010)**.

Egypt as one of the developing countries has no disease registry except for some common infectious disease such as HCV. Inflammation bowel disease (IBD); as one of autoimmune background disease is considered rare in Egypt, consequently has not been officially registered. Recently, the awareness to the IBD diagnosis and management in Egypt and the Middle East is increasing **(El-Atrebi and El-Bassyouni, 2017)**.

The etiopathogenesis of IBD is not yet fully elucidated, but it is known to involve the interaction between four major components: an aberrant immune system, genetic factors, environmental factors, and intestinal microbiota (therefore, the presence of only one component does not cause the onset of IBD) **(De Souza and Fiocchi, 2016)**. The inflammatory response is mediated by immune cells (T-helper 1 and T-helper 17 in CD and T-helper 2 in UC), cytokines (Tumor Necrosis Factor-α (TNF-α), transforming growth factor-β, and interleukins- (IL-) 12, IL-17, and IL23), chemokines, reactive oxygen species, neuropeptides, and nonimmune (myeloid, epithelial, mesenchymal, lymphoid, neurogenic, and endothelial) cells **(Neurath, 2014; Coskun et al., 2017)**.

The primary immune response to one or more stimuli induces tissue destruction and proliferation of endothelial and mesenchymal cells, resulting in a secondary immune response that amplifies the already present inflammation and stimulates fibrosis, tissue remodeling, angiogenesis, and lymphangiogenesis **(De Souza and Fiocchi, 2016)**. The nonresolving inflammation determines the installation of a vicious cycle of self-sustaining