عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	نمط التعبير الجيني والمثيلة في الجينات الكابتة لسرطان القولون والمستقيم
Title of the proposed project in English	Methylation and gene expression pattern of tumor suppressor genes in colorectal cancer
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التخصص الدقيق للمشرف الرئيس - Specialty of PI	Molecular Biology- DNA repair
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المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا بالشهور)) - Expected time in month to finish	12
Abstract of the proposal (No more than 200 words)	Colorectal cancer, a leading cause of mortality worldwide, is a multistep disorder that results from the alteration of genetic and epigenetic mechanisms under contextual influence. Epigenetic aberrations, including DNA methylation, histone modifications, chromatin remodeling and non-coding RNAs, affect every aspect of tumor development from initiation to metastasis1. Genetic changes, such as mutations in proto-oncogenes and DNA repair genes, and loss of function in the tumor suppressor genes cause colorectal cancer development. Abnormal DNA methylation is also known to play a crucial role in colorectal carcinogenesis2. Colorectal cancer undergoes the adenoma-carcinoma sequence or de novo pathway, and multiple steps of genetic changes such as the loss or mutation of adenomatous polyposis coli (APC), alteration of DNA methylation, chromosome loss, mutation of the p53 gene and/or mutation of the PTEN gene occur sequentially3. Aberrant DNA methylation of CpGs in the proximity of predicted transcription start sites (TSS) often leads to alterations in gene function and pathway deregulation in human cancer. It has been increasingly shown over the past 10 years that the CpG islands in the promoter regions of a large number of genes, which are mostly unmethylated in normal tissues, are methylated to varying degrees in human cancer2. Since aberrant gene methylation is one of the earliest molecular alterations

	occurring during cancer, it has emerged as a promising strategy for the early detection of cancer. Epigenetic events linked to tumor suppressor gene inactivation through promoter methylation, are more frequent events than somatic mutations in cancer, and may be driving tumorigenic initiation and progression3,4,5. Promoter methylation of tumor suppressor gene have previously been identified and characterized in several cancers. Therefore, in the present study, we will investigate gene expression pattern and aberrant methylation of promoter regions in three tumor suppressor genes (APC, P53, and PTEN) in colorectal cancer tumor samples. The association between the methylation status and gene expression patterns of these genes and major clinicopathological parameters of patients will also be evaluated.
Hypothesis of the proposal	Similar to other tumor types, colorectal cancer is thought to arise following the activation of oncogenes and inactivation of tumor suppressor genes. In addition to genetic alterations, epigenetic abnormalities, such as changes in genomic DNA methylation patterns, are associated with all human malignancies. Changes in the DNA methylation pattern in cancer include global hypomethylation of the CpG dinucleotides in repetitive DNA regions in conjunction with hypermethylation of CpG island promoter-associated genes. In colorectal cancer, a growing number of genes have been recognized as undergoing aberrant methylation at CpG islands, suggesting this to be an important molecular mechanism in the development of colorectal cancer. However, most of these studies have focused on a single candidate gene and the reported frequencies and disease specificities vary between independent studies. These discrepancies most likely reflect differences in the populations that were studied and the methods used. Previous studies have suggested that methylation profiles of cancers are tumor type- and ethnicity-specific and very few reports are available on Middle East population. Hence in the present study we are intended to screen the methylation and expression patterns of key tumor suppressor genes in Saudi colorectal cancer patients.
Specific objectives	1. Evaluation of expression levels of APC, P53, and PTEN gene in normal versus cancerous tissues using Q-RT-PCR and immunohistochemistry (IHC). 2. To investigate the methylation status of APC, P53, and PTEN and correlate findings with gene expression and clinicopathological parameters.
	Patient samples: Colorectal tissue and blood samples from Saudi patients will be obtained from the collaborators and

Methodology & Major Techniques to be used

clinicians as per the guidelines of IRB. Patients attending the oncology department at KKUH will be examined by the oncologist and routine examination performed. Patient group would comprise men and women with colorectal cancer of all ages and stages of the disease. Surgical core biopsy and adjacent normal specimen will be obtained prior to treatment and immediately stored in RNAlater solution (Ambion) for DNA and RNA extraction. 5 ml of blood will also be collected from each patient as a control. Formalin-fixed paraffin blocks of patient tissue samples will be obtained from the pathology lab for immunohistochemical studies. Informed consent from all the study participants will be obtained. Nucleic acid Isolation: High–molecular-weight DNA/RNA will be obtained from freshly collected placenta samples and blood samples (DNA) using Qiagen DNA/RNA mini prep kit and Qiagen nucleic acid extraction kits according to the manufacturer's protocol. Quantitative RT–PCR for MGMT: Expression will be assessed by quantitative RT–PCR in triplicate using specific primers for tumor suppressor genes APC, P53, and PTEN. The relative amount of RNA will be calculated with the CT method. Gene expression will be normalized with the GAPDH gene, and the level of expression of the tumour sample will be compared with the mean level of the gene expression in normal colon tissues and expressed as an n-fold ratio. Immunohistochemistry: IHC for APC, P53, and PTEN genes will be performed in representative colon tumour and normal tissue. Briefly, deparaffinized and rehydrated sections will be subjected to microwave treatment in 10mM sodium citrate buffer, pH 6.0, for antigen retrieval. The sections will be incubated overnight at 4° C in a humidified chamber with the primary antibody of APC, P53, and PTEN (sc-28241, dilution 1:100; Santacruz Biotechnology Inc., USA). The detection will be performed with a labelled streptavidin–biotin immunoperoxidase detection system and the immunohistochemical staining will be developed with 3, 30-diaminobenzidine substrate. Omission of the primary antibody incubation will be used as negative control. Methylation Analysis: DNA methylation status in the CpG island of APC, P53, and PTEN genes will be evaluated by PCR analysis of bisulfite modified genomic DNA, which induces chemical conversion of unmethylated, but not methylated, cytosine to uracil. DNA (1 µg) will be modified with sodium bisulfite using the EZ DNA methylation-gold kit (Zymo Research, CA, USA) according to the manufacturer's instructions. Methylation-specific polymerase chain reaction (MSP) will be performed using primers specific for methylated and unmethylated DNA. In vitro methylated DNA were will be used as a positive control for the methylation of APC, P53, and PTEN genes and DNA from normal tissues will be used as a negative control. Methylation pattern will be screened

	using agarose gel electrophoresis.
Availability of Samples	Yes
Availability of Chemicals	Yes
Availability of Instruments	Yes
Ethical Approval	Ethical approval is available
Recent References	1. Jin, Bilian, and Keith D. Robertson. "DNA methyltransferases, DNA damage repair, and cancer." Epigenetic Alterations in Oncogenesis. Springer New York, 2013. 3-29. 2. OZDEMIR, F., ALTINISIK, J., KARATEKE, A., COKSUER, H., & BUYRU, N. (2012). Methylation of tumor suppressor genes in ovarian cancer. Experimental and Therapeutic Medicine, 4(6), 1092–1096. 3. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. science. 2013 Mar 29;339(6127):1546-58. 4. Cancer Genome Atlas Network, 2012. Comprehensive molecular characterization of human colon and rectal cancer. Nature, 487(7407), pp.330-337. 5. Hinoue, Toshinori, et al. "Genome-scale analysis of aberrant DNA methylation in colorectal cancer." Genome research 22.2 (2012): 271-282.