عنوان المشروع باللغة العربية	واستجابة الحمض POTالتيلومير و TRF1 من sheltrin دور مكونات التيلومير النووي من التلف في تطور سرطان القولون والمستقيم في المرضى السعوديين
عنوان المشروع باللغة الإنجليزية	The Role of telomere sheltrin components of telomere TRF1 and POT and DNA damage response in colorectal cancer progression in Saudi patients
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التخصص الدقيق للمشرف الرئيس	Proteins and Molecular Biology
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المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا	12 months
Abstract or synopsis of the proposal (200 words or less):	Colorectal cancer in Saudi Arabia have become a series issue in the last few years, which is the number one cancer leading to death. CRC is a heterogeneous disease, an d it could be caused by environmental and genetics factors. At the molecular level, many changes occurs in CRC facilitating its progression. Telomeres are nucleoprotein complexes located at ends of chromosomes which function to protect chromosome ends and ensure their complete replication. Human telomeres are composed of TTAGGG tandem repeats in addition to groups of proteins called shelterin complex, which protects chromosome ends, regulates telomere length, recombination, and DNA damage checkpoints. Shelterin is composed of TRF1, TRF2, POT1, TPP1, TIN2, and RAP1. The loss of telomere protection is the root cause of the premature aging symptoms associated with Dyskeratosis congenita and other telomeropathies. Furthermore, telomere dysfunction plays an important role in the early stages of cancer and the activation of a telomere maintenance system (telomerase or ALT) is a hallmark of human cancer. Normally, TRF1 and POT1 have major roles in the interaction of telomeres with the DNA damage signaling machinery. TRF1 and POT1 have been shown to contribute to telomere length regulation and suppresses DNA breakage at TTAGGG repeats under replicative stress, a phenomenon described as telomere fragility and the loss of TRF1 results in telomere replication errors and the activation of ATR signaling in S-phase. On the other hand, when ATR signaling is inhibited, dysfunctional chromosome ends were not detected as damage. Recently, evidence is accumulating that links altered shelterin function with human cancer. The aim of this study is to determine how sheltrin complex is regulated in cancer and how it affect ATR, which is an important protein in DDR, this will be done by the expressional level of (TRF1, POT1, ATR and chk1) and protein level determination using immunohistochemistry.
Hypothesis or	Telomeres are considered anti-cancer targets, as telomere

scientific justification of the proposal	maintenance above a minimum length is necessary for cancer growth. Telomeres offer distinct advantages for the study of the DDR. They represent molecularly marked sites in the genome that can be converted to sites of DNA damage by manipulation of shelterin. As a result, their structure and behavior can be studied before and after the induction of DNA damage signaling. The functions of telomeric repeat-binding factor 1 (TRF1) in colorectal cancer are largely unexplored. This study examined the relationship between the expression of TRF1, POT1 and ATR and clinicopathological variables. Using these telomeric tools, we can gain insight into several aspects of the DNA damage response in colorectal cancer.
Specific objectives	1. Determining the expressional level of (TRF1, POT1, ATR and chk1) in colorectal cancer and its matched control 2. Protein level determination using Immunohistochemistry of TRF1, POT1, ATR and chk1 3. Telomere length determination in colorectal cancer tissue relative to its matched control
Methodology & Major Techniques to be used	Patient samples: Colorectal tissues from Saudi patients will be obtained from the collaborators and 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 (n=20). 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. Nucleic acid Isolation: High–molecular-weight DNA/RNA will be obtained from freshly collected colorectal cancer samples, matched normal 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: Expression will be assessed by quantitative RT–PCR in duplicate using TaqMan (Applied Biosystems) gene expression assays for TRF1, POT1, ATR and chk1. The relative amount of RNA will be calculated with the CT method. Gene expression will be normalized with the RNA ribosomal 18S, and the level of expression of the tumor sample will be compared with the mean level of the gene expression in normal liver tissues and expressed as an n-fold ratio. Immunohistochemistry: IHC for TRF1 and POT1 will be performed in representative colon tumor 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 TRF1 and POT1. 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. Relative Telomere length Determination: Telomere length measurement of CRC and their matched normal tissues will carried out using RT-PCR.
Availability of Samples	YES
If the answer is no, kindly justify	
Availability of Chemicals	YES
If the answer is no, kindly justify	
Availability of Instruments	YES
Availability of Ethical Approval (if needed)	YES
Recent References	• Michal Zimmermann, Tatsuya Kibe, Shaheen Kabir, and Titia de Lange. 2014. TRF1 negotiates TTAGGG repeat associated replication problems by recruiting the BLM helicase and the TPP1/POT1 repressor of ATR signaling. GENES & DEVELOPMENT 28:2477–2491. • Hui-Ching Chuang, Chang-Han Chen, Chao-Cheng Huang, Fu-Min Fang, Hsin-Ting Tsai and Chih-Yen Chien. 2011. Reduced expression of TRF1 is associated with tumor progression and poor prognosis in oral squamous cell carcinoma. Experimental and Therapeutic Medicine 2: 63-67. • María García-Beccaria, Paula Martínez et.al. 2015. Therapeutic inhibition of TRF1 impairs the growth of p53-deficient K-RasG12V - induced lung cancer by induction of telomeric DNA damage. EMBO Molecular Medicine Vol 7: No 7. • Baumann, P., and T. R. Cech. 2001. Pot1, the putative telomere end-binding protein in fission yeast and humans. Science 292:1171–1175. • Barrientos, K. S., M. F. Kendellen, B. D. Freibaum, B. N. Armbruster, K. T. Etheridge, and C. M. Counter. 2008. Distinct functions of POT1 at telomeres. Mol. Cell. Biol. 28:5251–5264.