عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	العلاقة بين تعدد الأشكال في منطقة long non coding RNA في جين PRNCR1 وخطر الإصابة بسرطان القولون والمستقيم في المجتمع السعودي
Title of the proposed project in English	Association between polymorphisms in long non-coding RNA PRNCR1 in 8q24 and risk of colorectal cancer in Saudi population
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التخصص الدقيق للمشرف الرئيس - Specialty of PI	Molecular Biology
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المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا (بالشهور) - Expected time in month to finish	8
Abstract of the proposal (No more than 200 words)	Colorectal cancer (CRC) is one of the most common cancers worldwide. Long non‐coding RNAs (lncRNAs) have been confirmed to play a critical regulatory role in various biological processes including carcinogenesis, which indicates that lncRNAs are valuable biomarkers and therapeutic targets. Prostate cancer non‐coding RNA 1 (PRNCR1), also known as PCAT8 and CARLo‐3, is a ~13 kb intron‐less lncRNA, which is transcribed from the 'gene‐desert' region of 8q24. It has been reported that PRNCR1 is associated with prostate cancer susceptibility and PRNCR1 could be involved in prostate carcinogenesis by modulating androgen receptor (AR) activity. This mechanism was further described by Yang et al. Binding of PRNCR1 to the acetylated AR and its association with DOT1L appear to be required for recruitment of a second lncRNA, PCGEM1, to the DOT1L‐mediated methylated of AR at the N-terminus. The interactions of these overexpressed lncRNAs may potentially serve as important regulators in prostate cancer. For CRC, many researchers have reported that the crucial locus of 8q24 may contribute to susceptibility to CRC. It has been gradually recognized that aberration of PRNCR1 might be a biological signature of CRC, but its specific expression pattern related to CRC remains unknown. The novel lncRNA prostate cancer non‐coding RNA 1 (PRNCR1) is located in the susceptible genomic area of CRC, however the functional role of PRNCR1 remains unknown in Saudi population. Previous results suggested that results suggest that individuals with PRNCR1 variants may have increased risk for developing cancer. Thus, we aimed to investigate the clinical significance of PRNCR1 in CRC.
	LncRNAs are RNA polymerase II-transcribed, polyadenylated, and frequently alternatively spliced RNAs with the features of cell-type specific expression. patterns, distinct subcellular

Hypothesis of the proposal	localizations, linkage to various diseases, and evolutionary selection of the lncRNA sequence. LncRNAs can be intergenic, intronic, antisense or overlapping with proteincoding genes or other ncRNAs. Recent studies have revealed the contribution of ncRNAs as protooncogene, tumor suppressor gene, drivers of metastasis transformation in cancer development. The expression of lncRNAs is deregulated in different cancers, including colon cancer. Several lines of evidence have shown that SNPs in lncRNAs may influence the process of splicing and stability of mRNA conformation, resulting in the modification of its interacting partners. In this study, we hypothesized that SNPs in lncRNAs may be involved in the risk of CRC. In the present study we are intended to investigate the association of PRNCR1 gene with colon cancer by evaluating PRNCR1 expression pattern and genetic polymorphisms.
Specific objectives	1. Identification and evaluation of deleterious SNPs in PRNCR1 gene that may predispose to colon cancer and to correlate this to expression. 2. Evaluation of expression levels of PRNCR1 gene in normal versus cancerous tissues using qRT‐PCR analyses.
Methodology & Major Techniques to be used	Patient samples: Colon cancer tissues from Saudi patients will be obtained from the collaborators and clinicians as per the guidelines of IRB. Patients attending the endoscopy department at KKUH will be examined by the oncologist and routine examination performed. Patient group would comprise women with colon cancer of all ages and stages of the disease (n=25). 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 for genotyping studies. 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. Genotyping: Genotyping of SNPs in PRNCR1 will be performed using TaqMan genotyping assays. The genotype data will be scored for further analysis. Deviation of the genotype frequencies of each SNP in the control subjects from those expected under the Hardy- Weinberg equilibrium (HWE) will be assessed by $\chi 2$ test. Odds ratios (ORs) were calculated and given with 95% confidence intervals (95% CI). Gene expression: One microgram total RNA will be reverse transcribed in a final volume of 10 µl using random primers under standard conditions using the PrimeScript RT reagent Kit. The relative levels of PRNCR1 will be determined by qPCR using gene specific primers. GAPDH will be used as an internal control, as its expression showed minimal variation in colorectal cancer samples. The PCR primers for PRNCR1 and GAPDH were as follows: PRNCR1 forward, 5'‐CCAGATTCCAAGGGCTGATA‐3' and reverse, 5'‐GATGTTTGGAGGCATCTGGT‐3' and GAPDH forward, 5′

	GTCAACGGATTTGGTCT GTATT 3′ and reverse, 5′ AGTCTTCTGGGTGGCAGTGAT 3′, ABI 7500 fast real-time PCR system (Life Technologies, Carlsbad, California, USA) will be used for these experiments. The Ct (threshold cycle) value of each sample will be calculated from the threshold cycles with the instrument's software (SDS 2.3), and the relative expression of PRNCR1 mRNA will be normalized to the GAPDH value. Data will be analyzed using the comparative threshold cycle (2-ΔCT) method.
Availability of Samples	Yes
Availability of Chemicals	Yes
Availability of Instruments	Yes
Ethical Approval	Ethical approval is available
Recent References	Yang, Liu, et al. "Upregulation of long non-coding RNA PRNCR1 in colorectal cancer promotes cell proliferation and cell cycle progression." Oncology reports 35.1 (2016): 318-324. Li, Lijuan, et al. "Association between polymorphisms in long non-coding RNA PRNCR1 in 8q24 and risk of gastric cancer." Tumor Biology 37.1 (2016): 299-303.