عنوان المشروع باللغة العربية	دور MEG3 , أحد أنواع الحمض النووي الرايبوزي الغير مصنع لبروتين في سرطان القولون
عنوان المشروع باللغة الإنجليزية	The role of Long non-coding RNA MEG3 in colorectal carcinoma
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التخصص الدقيق للمشرف الرئيس	Molecular Biology
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المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا	شهر 12
Abstract or synopsis of the proposal (200 words or less):	Colon cancer is a multifactorial disease that progresses through a series of well-defined premalignant stages due to mutations and epigenetic factors. Long non-coding RNAs (lncRNA) are a class of RNA molecules arbitrarily defined as being longer than 200 nucleotides and not translated into a protein. Initially, it was thoug ht that lncRNAs represent transcriptional noise, but it is nowadays recognized that lncRNAs may have biological roles. Maternally expressed gene 3 (MEG3), an lncRNA, is expressed in many normal tissues. However, MEG3 expression is lost in an expanding list of primary human tumors, and promoter hypermethylation or hypermethylation of the intergenic differentially methylated region has been shown to contribute to the loss of MEG3 expression in tumors. MEG3 represents as a tumor suppressor gene, and its ectopic expression can inhibit cell proliferation and promote cell apoptosis in human glioma cell lines. Moreover, accumulation of p53 (TP53) and its target gene expression partly contribute to cell growth inhibition induced by MEG3. In previous studies Lu et al.1 suggested that MEG3 possesses tumour suppressive function mediated by p53-dependent and p53-independent pathways, and Yan et al. 2 reported that promoter hypermethylation down regulates MEG3 expression and promotes onc ogenesis. DNA methyltransferase 1 (DNMT1) gene has been reported to play a key role in MEG3 promoter methylation and its expression (Anwar et al. 3, He et al. 4). In this study we are aiming to examine the expression (MEG3, TP53 and DNMT1) and DNA methylation pattern of MEG3 and TP53 genes in Saudi colorectal cancer, to evaluate role of MEG3 in tumor progression.
Hypothesis or scientific justification of the proposal	In recent years, long non-coding RNAs (lncRNAs), which are currently defined as transcripts of greater than 200 nucleotides without evident protein coding function, have drawn more and more attention in several diseases. MEG3 a long noncoding RNA possesses tumour suppressive function mediated by p53-dependent and p53-independent pathways. It is expressed in many normal human tissues and recent studies have demonstrated the decreased e xpression of MEG3 in several tumors such

	as hepatocellular carcinoma, lung cancer, bladder cancer and kidney cancer, which is associated with development and progression of cancer. Moreover, some evidences have shown that hypermethylation of differentially methylated regions plays a major role in the silence of MEG3 gene in tumors. These data suggest that MEG3 may play an important role in a specific pathway of tumor pathogenesis, but the exact mechanism of the low expression of MEG3 is still poorly understood. MEG3 expression has not been studies in colorectal cancer. In the present study we are evaluating expression pattern of MEG3, TP53 and DNMT1 and methylation status of MEG3 and TP53 genes and their association with colorectal cancer risk in Saudi population.
Specific objectives	The main aims of the present study are: 1. To examine the expression pattern of MEG3, TP53 and DNMT1 genes in colorectal cancer and comparing the expression levels with clinicopathological conditions. 2. To elucidate the effect of DNA methylation on the expression of MEG3 and TP53 genes and their association with colorectal cancer in Saudi population.
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 will be performed. Patient group would comprise men and women with colorectal cancer of all ages and stages of the disease (n=40). 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. Nucleic acid and miRNA Isolation: High–molecular-weight genomic DNA will be obtained from freshly collected colorectal cancer samples, matched normal samples using Qiagen DNA/RNA mini prep kit kits according to the manufacturer's protocol. Quantitative RT-PCR: 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 MEG3, TP53 and DNMT1 will be determined by qPCR using gene specific primers. GAPDH was measured as an internal control, as its expression showed minimal variation in colorectal cancer samples. The PCR primers for MEG3, TP53 and GAPDH were as follows: MEG3 sense, 5′ CTGCCCATCTACACCTCACG 3′ and reverse, 5′ CTCTCCGCCGTCTGCGCTAGGGGCT 3′; p53 sense 5′ CCAGGGCAGCTACG-GTTTC3′, p53- reverse 5′ CTCCGTCATGTGCTGTGACTG, GAPDH sense, 5′ GTCAACGGATTTGGTCT GTATT 3′ and reverse, 5′ AGTCTTCTGGGTGGCAGTGAT 3′, DNMT1 sense CCATCAGGCATTCTACCA and reverse CGTTCTCCTTGTCTTCTCT. 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 MEG3, TP53 and DNMT1 mRNA will be normalized to the GAPDH value. Data will be analyzed using the comparative threshold cycle (2-ΔCT) method. Methylation: Methylation-specific (MS)-PCR will be used for qualitative DNA methylation analysis of MEG3 and TP53 gene promoter regions. Genomic DNA will be treated with bisulphate and methylation status will be analysed using MS-PCR method. Primers will be designed to detect methylated DNA (M primers) and unmethylated DNA samples. Techniques to be used in the project: • cDNA preparation • RT-PCR for Gene expression • Methylation specific 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	1. Lu, Kai-hua, et al. "Long non-coding RNA MEG3 inhibits NSCLC cells proliferation and induces apoptosis by affecting p53 expression." BMC cancer 13.1 (2013): 461. 2. Yan, Jiang, et al. "MiR-148a regulates MEG3 in gastric cancer by targeting DNA methyltransferase 1." Medical Oncology 31.3 (2014): 1-7. 3. Anwar, Sumadi Lukman, et al. "Loss of imprinting and allelic switching at the DLK1-MEG3 locus in human hepatocellular carcinoma." (2012): e49462. 4. He, Yong, et al. "Long noncoding RNAs: Novel insights into hepatocelluar carcinoma." Cancer letters 344.1 (2014): 20-27.