عنوان المشروع باللغة العربية	دور التعبير الجيني و التغييرات الجينية لإنزيم thymine DNA glycosylase في نشوء عدم إستقرار الجينوم عند مريضات سرطان الثدى
عنوان المشروع باللغة الإنجليزية	Role of Thymine DNA Glycosylase gene expression and polymorphism in genomic instability of breast cancer patients
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التخصص الدقيق للمشرف الرئيس	Molecular Biology- DNA Repair
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المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا	10 months
Abstract or synopsis of the proposal (200 words or less):	Thymine DNA glycosylase (TDG) is a monofunctional DNA glycosylase that functions in base excision repair (BER), the pathway responsible for repairing up to 20,000 endogenous lesions/cell/day. This glycosylase is well known for its ability to remove T from G:T mispairs and can also excise a variety of other bases, some of which include U opposite A and 5-fluorouracil (5- FU) paired with A or G. More recent work has implicated TDG in an active demethylation pathway with the ten-eleven translocation (TET) protein family. It has been shown both biochemically and biologically that TDG can remove TET generated 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) in a process that requires BER to regenerate unmodified C. Because both DNA repair and DNA methylation dynamics are vital processes to the maintenance of genomic stability, aberrant activity of either of these processes could contribute to cancer development. The rs4135113 single nucleotide polymorphism (SNP) of TDG is found in 10% of the global population. This coding SNP results in the alteration of Gly199 to Ser199Gly is part of a loop responsible for stabilizing the flipped abasic nucleotide in the active site pocket. Previous re sults suggested that results suggest that individuals with TDG variants may have increased risk for developing cancer.
Hypothesis or scientific justification of	Thymine DNA glycosylase (TDG) functions in base excision repair, a DNA repair pathway that acts in a lesion-specific manner to correct individual damaged or altered bases.

the proposal	Recent work found that TDG expression levels are upregulated in few cancers and that TDG serves to regulate Wnt signaling, a key driver for cancer. Interestingly, depletion of TDG significantly inhibited cancer cell proliferation and tumor formation in this study, suggesting TDG is required for cancer growth and may serve as a biomarker. There is limited available evidence on TDG polymorphisms in relation to cancer or biomarkers of cancer. One study found G199S to be associated with increased likelihood of mic ronuclei in Chinese workers who had been exposed to vinyl chloride, suggesting individuals carrying G199S are more susceptible to chromosomal damage. In the present study we are intended to investigate the association of TDG gene with breast cancer by evaluating TDG expression pattern and genetic polymorphisms.
Specific objectives	1. Evaluation of expression levels of TDG genes in normal versus cancerous tissues using immunohistochemistry. 2. Identification and evaluation of deleterious SNPs in TDG gene that may predispose to breast cancer and to correlate this to expression.
Methodology & Major Techniques to be used	Patient samples: Breast cancer 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 women with Breast 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 breast 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 TDG 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 χ2 test. Odds ratios (ORs) were calculated and given with 95% confidence intervals (95% CI). Immunohistochemistry: IHC for Thymine DNA glycosylase 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 TDG. 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.
Availability of Samples	YES
Availability of Chemicals	YES
Availability of Instruments	YES
Availability of Ethical Approval (if needed)	YES
Recent References	1. Sjolund A, Nemec AA, Paquet N, Prakash A, Sung P, et al. (2014) A Germline Polymorphism of Thymine DNA Glycosylase Induces Genomic Instability and Cellular Transformation. PLoS Genet 10(11): e1004753. doi:10.1371/journal.pgen.1004753 2. Kohli, R. M., & Zhang, Y. (2013). TET enzymes, TDG and the dynamics of DNA demethylation. Nature, 502(7472), 472-479.