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عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	استهداف جزيئات RNA اللاجينية المتعدده في علاج سرطان الثدي باستخدام مركب الأنيثول
Title of the proposed project in English	Targeting the profile of multiple lncRNAs involved in breast cancer treatment by Anethole
المشرف الرئيس - PI	**عبيرعبد القادر الغنوشي**
التخصص الدقيق للمشرف الرئيس - Specialty of PI	البروتينات و الكيمياء الحيوية الكلينيكية
المشرف المساعد - Co-PI	**عبد الحبيب السملالي**
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)	Cancer has been the leading cause of mortality worldwide with 14.1 million new cases and 8.2 million deaths in 2012 (Siegel et al., 2017). However, the second leading cause of death in women is breast cancer, which is characterized by diverse genomic and transcriptomic profiles. Although current chemotherapeutics as well as modern targeted therapy-approaches have been developed against breast cancer, the response rates to these agents remain low; the magnitude of tumor regression is variable and transient. De novo and acquired resistance to chemotherapeutics and targeted therapies and the toxicity to normal cells are the major causes of treatment failure. Therefore, it is necessary to search for new and better treatments for breast cancer. To these end, attention could be drawn toward phytochemicals derived from folk medicine due to their safety. Anethole, a major constitutent of Foeniculum vulgare (fennel) essential oil, is widely used in traditional medicine. It possesses wide pharmacological actions and, importantly, has low toxicity which led the support to the rationale behind its therapeutic uses. Anethole has been found to exhibit anti-oxidant and anti-inflamatory activities, to inhibit the proliferation of various tumor cells in culture and to prevent carcinogen induced tumors in rodents. Anethole'santicarcinogenic, anti-inflammatory, and growth-modulatory effects have been ascribed to the deactivation of NF-κB and AP-1 and their associated signaling molecules. Though a number of critical studies have been conducted to identify the underlying mechanisms that account for breast cancer treatment by Anethole, little is known regarding the biological role of long non-coding RNA (lncRNA) in this process. Distinct mRNA expression signatures discriminate breast cancer subtypes with different clinical implication. Despite, the mammalian genome is expressed in numerous non-coding transcripts which are specifically expressed through cell development, tumorigenesis, and metastasis (Reiche et al., 2014). Moreover, noncoding RNAs (ncRNAs) have been found as crucial molecules playing diverse regulatory roles in development and disease including cancer (Fatima et al., 2015). LncRNAs are a class of

	transcripts longer than 200 nucleotides with extremely limited protein coding potential (Kapranov et al., 2007). They represent, at least, four times more than coding RNA sequences. Recently, many studies have demonstrated that lncRNAs had multiple functions in a wide range of biological process, including, proliferation, apoptosis, cell migration, and cell invasion (Ponting et al., 2009; Sun et al., 2014). Recently, abnormal expression of lncRNAs have been realized to have a critical role in tumorigenesis, with numerous hundred lncRNAs dysregulation has been associated with the development and progression of several cancer types, which makes them potent biomarkers for cancer diagnosis and prognosis (Bolha et al., 2017; Heery et al., 2017; Ning et al., 2016; Prensner and Chinnaiyan, 2011,). Although lncRNAs have been reported to modulate tumor metastasis, the biological roles of lncRNAs in the process of Anethole treatment in breast cancer are not yet studied.
Hypothesis of the proposal	Because of its substantially wide range of activities, Anethole would allow targeting multiple molecular and cellular pathways involved in the process of breast cancer carcinogenesis. The data generated by this study may lead to the design of more effective drug against breast cancer.
Specific objectives	This project aims to: 1- investigate the lncRNAs dysregulation by Anethole in breast cancer cells and in breast cancer animal model by qPCR- Array. 2- establish the link between the selected lncRNAs deregulated by Anethole and breast cancer progression by quantitative reverse transcription PCR method. 3- investigate the effect of the over/ down-expression of lncRNAs selected on the Anethole effect (Breast cancer cells proliferation , migration and apoptosis)

Methodology & Major Techniques to be used	1- Breast cancer Cell culture The MCF-7 and MDA322 cells will be cultured in Dulbecco's Modified Eagle's Medium containing 10% heat-inactivated fetal bovine serum albumin in 95% humidified incubator with 5% CO2 at 37C with penicillin–streptomycin (10,000 U/mL). 2- lncRNA extraction Total lncRNA will be isolated from the breast cancer cells using Qiagen Kit for lncRNA according to the manufacturer's instructions. To quantify the lncRNA concentration and purity, we will use a NanoDrop8000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) 3- Reverse transcription cDNA synthetizes will be used from 1 µg of each Establish the link between the selected lncRNAs deregulated by Anethole and breast cancer progression by quantitative reverse transcription PCR method using cDNA reverse transcription kit from Applied Biosystems (Foster City, CA, USA). 4- Detection of deferentially expressed lncRNA by quantitative real-time PCR array as previously described by Semlali et al. (2011) 5- Patients and specimens The present study will be a retrospective analysis which includes 20 patients with primary breast cancer newly diagnosed from July 2015 to July 2017 at King Khaled Hospital. This retrospective analysis was approved by the Research Ethics Committee of King Khaled Hospital. All patients voluntarily joined this study and signed informed consents. The breast cancer blots tissues collected by for each patient to analyze the miRs levels using the qRT-PCR method. 6- qRT-PCR: The lncRNA will be isolated from breast cancer and normal breast tissue samples by specific kit from Qiagen. The complementary DNA will be synthesized reverse Transcriptase Kit (Thermo Fisher Scientific). The quantitative analysis of the lncRNA selected by PCR Array level will be performed using qRT-PCR, and GAPDH will be used as an endogenous control. qRT-PCR will be

	conducted using the Applied Biosystems 7500 Fast real-time PCR detection system. The fold changes will be calculated through the relative quantification using $2-\Delta\Delta Ct$. All the reactions will be performed in a 20 µL reaction volume in triplicate. 7- In situ hybridization analysis: Breast cancer tissues for in situ hybridization (ISH) will be fixed in 4% paraformaldehyde solution, dehydrated in a graded series of ethanol baths, and embedded in paraffin. Then, the tissue slides will be deparaffinized and digested with proteinase K for 30 minutes. After that, the slides will be prehybridized in a hybridization solution at 57°C for 2 hours. Tissues will be hybridized overnight in the presence of 10 ng 3′-5′biotin- labeled lncRNA selected (Exiqon) probes at 63°C. Slides will be washed twice stringently and an immunological reaction will be carried out by using the rabbit antibody against biotin and alkaline phosphatase, according to the manufacturer's recommendation. Each side will be assigned a score for intensity and staining positive pattern. 8- Proliferation by MTT assay The Check2 plasmid (Promega Corporation, Fitchburg, WI, USA) will be used for the over-expression of the lncRNAs selected. The proliferation will be evaluated by MTT assay after stimulation by anethole for 12h, 24h and 48h according to Semlali et al. (2011). 9- Apoptosis assay by Annexin and Pi test: The apoptosis effect of over-expression of the lncRNAs selected, on MCF-7 and MDA-MB-231 cell lines will be quantitatively assessed by Annexin V and Pi detection kit and analyzed by flow cytometry with proper machine settings. 10- Statistical analysis: the data will be expressed as mean ± standard deviation of at least three independent experiments. One-way analysis of variance will be used to test the differences between the mean values. All P-values will be two sided, and a value of 0.05 will be considered to be statistically significant. All statistical calculations will be performed using Statistical Package for the Social Sciences software (version 18.0; SPSS Inc., Chicago, IL, USA).

Availability of Samples	Yes
Availability of Chemicals	Yes
Availability of Instruments	Yes
Ethical Approval	Ethical approval is available
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