عنوان المشروع باللغة العربية	مقارنة تأثيرات الترانسكربتومكس على الجينات المختلفة في أنسجة مختلفة (من الفئران العادية والفئران المعالجه بالجفتنب (دواء لمعاجة السرطان
عنوان المشروع باللغة الإنجليزية	Cross omics comparison of effects on various genes in different tissues of normal mice and mice treated with gefitinib (anticancer drug)
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المشرف المساعد	د/سلمى الوكيل
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا	12 months
Abstract or synopsis of the proposal (200 words or less):	Anticancer drugs have distinct mechanisms of action which may vary in their effects on different types of normal and cancer cells. A si ngle "cure" for cancer has proved elusive since there is not a single type of cancer but there are as many as 100 different types of existing cancers with severe clinical manifestation [1]. In addition, there are very few demonstrable biochemical differences between cancerous cells and normal cells. For this reason the effectiveness of many anticancer drugs is limited by their toxicity to normal rapidly growing cells. A number of angiogenic factors and their pathways have been described, some of which have proven as therapeutic targets, such as PDGF/PDGFR (platelet-derived growth factor/receptor) and VEGF/VEGFR (vascular endothelial growth factor/receptor) [2] and [3]. Many anti-VEGF agents are used to treat neoplastic and nonneoplastic diseases [4] and [5]. Antiangiogenic receptor tyrosine kinase inhibitors (RTKIs) are widely used in the treatment of cancer [6] and [7]. RTKIs target multiple receptors that broadly inhibit angiogenesis. A receptor tyrosine kinase inhibitor i s a pharmaceutical drug that inhibits tyrosine kinases. Tyrosine kinases are enzymes responsible for the activation of many proteins by signal transduction cascades. The proteins are activated by adding a phosphate group to the protein (phosphorylation), a step that TKIs inhibit. TKIs are typically used as anticancer drugs. Numerous TKIs aiming at various tyrosine kinases have been generated. For example gefitinib and erlotinib aiming at the epidermal growth factor receptor. The epidermal growth factor receptor (EGFR) and the EGF-family of peptide growth factor have a central role in the pathogenesis and progression of different carcinoma types. The EGF ligand/receptor system is also involved in early embryonic development and in the renewal of stem cells in normal tissues such as the skin, liver and gut However, it is important to emphasize that the EGFR belongs to a family of receptors that encompasses three additional proteins, ErbB-2, ErbB-3 and ErbB-4. The mechanism of action of EGFR inhibitors is the inhibition of tumor cell proliferation and induction of apoptosis. The addition of EGFR inhibitors to standard chemotherapy is an attractive approach to enhance its efficacy. EGFR is overexpressed in the cells of certain types of human carcinomas - for example in lung and breast cancers. This leads to inappropriate activation of the anti-apoptotic Ras

	signalling cascade, eventually leading to uncontrolled cell proliferation. Research on gefitinib-sensitive non-small cell lung cancers has shown that a mutation in the EGFR tyrosine kinase domain is responsible for activating anti-apoptotic pathways [8] [9]. These mutations tend to confer increased sensitivity to tyrosine kinase inhibitors such as gefitinib and erlotinib. Of the types of non-small cell lung cancer histologies, adenocarcinoma is the type that most often harbors these mutations. These mutations are more commonly seen in Asians particularily women, and non-smokers (who also tend to have a high risk of having adenocarcinoma). In August 2013, the BBC reported that researchers in Edinburgh and Melbourne found, in a small-scale trial of 12 patients, that the effectiveness of Methotrexate for treating ectopic pregnancy was improved when Gefitinib was also administered [10].
Hypothesis or scientific justification of the proposal	Gefitinib has many side effects on human body and till date there is no knowledge on expression pattern of human transcriptome. The difference in pattern of genes of mice treated with gefitinib may reflect the differential pattern of expression in humans if any homology in differentially expressed genes will be found in both species. Identification of underlying genes and associated will shed new light on the side effects caused by the drug
Specific objectives	1. Normal Expression patterns in mouse 2. Transcriptome analysis on mouse administered Gefitinib dissolved in 10% methanol (75mg/kg) single ip injection for 24 h 3. Transcriptome analysis on mouse administered 10%methanol single ip injection for 24h 4. Perform functional studies to characterize differentially expressed genes and/or proteins.
Methodology & Major Techniques to be used	Collection of tissues at various intervals Treatment of mice with gefitinib dosing for the initial week of treatment was 75 mg/kg bw for five consecutive days followed by two days without treatment. Sample collection and nucleic acid (DNA/RNA) extraction Genomic DNA will be extracted using the Gentra Systems PUREGENE DNA Isolation kit. DNA will be quantified spectrophotometrically and then stored at -70ºC until required. PaxGene tubes will be stored at -80oC until required for RNA extraction. All procedures will essentially be in accordance with the manufacturers recommendations. Gene Expression with mice array All the samples would be grouped and the samples would be carried out for the global gene expression in triplicates and total RNA will be extracted using Trizol Standard methods. The quality and yield of RNA will be assessed using am Agilent 2100 Bioanalyser (Agilent Technologies, Santa Clara, California). Samples yielding RNA Integrity Number (RIN) equal or greater than 8 are to be considered and two replicates of each sample will be run on Affymetrix GeneChip Mouse Genome U133 Arrays (Affymetrix, Santa Clara) according to manufacturer's instructions

	Briefly, total of 200 ng of RNA will be reverse transcribed to synthesize first strand of cDNA with the help of oligo dT primers containing T7 flanking sequence. Following second strand synthesis, invitro transcription step reaction was carried with labelled aRNA. Following purification and fragmentation of RNA, the samples would be hybridized overnight onto U133 mouse genome arrays. Finally after washing to remove the unbound transcripts, the hybridized microarrays will be scanned and the intensity (cel) files with the acquisition and initial quantification of array images would be generated using Expression Console 1.3. Data Analysis We will use RMA analysis for quality control to filter and eliminate genes displaying an averaged intensity inferior to the global array background. For subsequent data analysis, cel files would be then imported into Partek Genomic Suite (Partek software v.6.3, Partek Inc., St. Louis, MO, USA) using the default normalization parameters to identify the differentially expressed genes between the various groups. Also to assess the differentially expressed genes in various dosage groups we will perform analysis for functional pathway, gene ontology and network analyses using Ingenuity Pathways Analysis (IPA). Transcipts/ genes displaying various significant overexpression and/or under expression will be validated accordingly. Real time PCR In order to validate our microarray results, confirmatory quantitative real-time RT-PCR (qRT-PCR) is performed using the ABI 7500 Sequence Detection System (ABI, Foster City, CA, USA). For this purpose, 50 ng total RNA procured from the same microarray study samples are transcribed into cDNA using Sensiscript Kit (QIAGENInc., Valencia, CA, USA) under the following conditions: 25 °C for10 min, 42 °C for 2 h, and 70 °C for 15 min in a total volume of 20 µl. The differentially expressed genes will be randomly selected and primers designed using Primer3 software. After primer optimization, the PCR assays will be performed in 6 µl of the cDNA using the QIAGEN Quantitet SyBR GreenKit, employing GAPDH as the endogenous control gene. All reactions will be conducted in triplicates and the data was analyzed using the delta delta CT method.
Availability of Samples	NO
If the answer is no, kindly justify	samples will be available after treatments
Availability of Chemicals	YES
If the answer is no, kindly justify	Not Applicable
Availability of Instruments	YES
Availability of Ethical Approval (if needed)	NO
Recent References	1. J. Folkman. Angiogenesis in cancer, vascular, rheumatoid and other

disease. Nat Med, 1 (1995), p. 27.

2. P. Carmeliet, R.K. Jain. Angiogenesis in cancer and other diseases. Nature, 407 (2000), p. 249

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