To: The Dean of Graduate Studies

From: PhD student
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Department: Human biology
Stage: (please sircle) **1st** Stage / **2nd** Stage

Annual Progress Report

Submission to the Graduate studies Authority by the departmental secretary only

Part A – designated for PhD Student

My research goals are to test whether expression of a gene called integrin beta 3 (Intβ3) in luminal A breast cancer cell lines can revert the cells back to a normal-like breast tissue when cultured in their relevant physiological microenvironment, thus normalizing their malignant phenotype.

To this end we addressed the following specific objectives:

1. Characterize human luminal breast cancer cells overexpressing Intβ3 for their cancer progenitor cell like properties.

2. Determine whether Intβ3 expression will induce malignant normalization of luminal breast cancer cells.

3. Determine whether knock down of Intβ3 in normal human breast cell line will disrupt their polarization.

4. Elucidate the molecular mechanisms by which Intβ3 expression in human luminal breast cancer cells promotes their differentiation to a normal like phenotype.

5. Evaluate whether overexpression of Intβ3 in luminal cancer cells will promote early dissemination of tumor cells *in vitro* and *in vivo*.

I have successfully carried out Aims 1, 3 and currently I am finalizing Aim 2 and Aim 4, and have started Aim 5. Our results demonstrate that cancer progenitor like cells expressing Intβ3, in MCF-7 (MCF-7-Intβ3) and T47D (T47D-Intβ3) breast cancer cell lines underwent phenotypic reversion resembling a normal-like acini when cultured *in vitro* in the physiological relevant 3 dimensional basement membrane extract (3D BME system), which is a novel model that recapitulates numerous features of breast epithelium *in vivo* (Aim 1 and 3). Importantly, the reversion of MCF-7-Intβ3 cells to a normal-like phenotype induced a dormant state when they cultured in the 3D BME model compared to MCF-7-vec cells (Aim 2). Thus, we wish to further study whether overexpression of Intβ3 in MCF-7 cells will promote their malignant normalization also *in vivo*. Intriguingly, we demonstrate that the reverted acini from the unique progenitor population in the

3D BME system resembled a pre-neoplastic stage (called usual ductal hyperplasia (UDH)) of breast tissue (Aim 2). This precancerous stage is characterized by an increase in the number of luminal cells within the ductal space without architectural distortion of the duct. Intriguingly, we find positive expression of Intβ3, which was confined to the polarized outer layer of luminal cells in all examined biopsy samples of UDH of luminal A breast cancer patients. Whereas, we could not detect Intβ3 expression in normal breast tissue as was reported previously and we rarely observed the expression of Intβ3 in both low and high-grade DCIS biopsy samples. Similarly, in invasive ductal carcinoma grade 1, we could rarely detect expression of Int-β3 in the tumor cells although we found some expression in the stromal cells. These surprising results are contradictory to previous reports suggesting that Int-αvβ3 plays a role in tumor progression. These findings demonstrate that the malignant phenotype can be normalized by reprogramming cancer luminal progenitor-like cells to differentiate via the expression of Intβ3.

Furthermore, we demonstrated that the reversion of MCF-7-Intβ3 cells to a normal-like phenotype was mediated by down-regulation of Notch4 expression and downstream signaling and can be partially reversed by inhibiting $\alpha_V\beta3$ activation (Aim 4). Therefore, we seek to further unravel the potential mechanisms that can be applied to redirect cancer luminal progenitor cells to commit and differentiate to a normal-like tissue, thus normalizing their malignant phenotype. This approach may lead to novel therapeutic strategy to treat recurring breast cancer disease.

Finally, we already have a preliminary results demonstrating that overexpression of Intβ3 in luminal cancer cells can promote early dissemination of tumor cells from the primary site in an in vitro and *in vivo* (Aim 5) model systems paradoxically resembling the invasive ductal carcinoma *in vivo*. Thus, the different luminal breast cancer cell lines overexpressing Intβ3 will be further tested for their metastatic potential *in vitro* and *in vivo*.

Estimated date for submitting PhD research proposal/ dissertation to the PhD departmental committee: April 2016

Ph.D. student Name: Hanan Abu Tayeh Signature: _____חנאן__ Date: 8.2.15

Part B - designated to the research the Chairperson of PhD Committee)

Please refer to the student progress and to the estimated date for submission of research proposal / dissertation.

Hanan has done an excellent job during this term and submitted a paper recently for publication. Hanan will submit her thesis on time April 2016 .

PhD research evaluation

(The evaluations relate to all researches previously supervised by you)

Evaluation	Poor	Average	Good	Very good	Ranked in the top 5%
Novelty					X
Clarity of research and hypothesis					X
Criticism				X	
Knowledge of Background material				X	
Knowledge of research methods					X
Comments					

Supervisors Name: _____Dalit Barkan_____ Signature: _____
Date:

Chairperson of PhD Committee Name: _____ Signature: _____ Date: _____