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Pathology and Pathogenesis of Metastasis in Novel Nude Mouse Models of Feline Mammary Cancer

A Thesis presented by

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For the Doctor of Philosophy Degree of Veterinary Science (PhD)

Pathology (General, Special and Postmortem)

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بسم الله الرحمن الرحيم

"وَقُل رَّبِّ زِدْنِي عِلْماً"

سورة طه (١١٣)

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Approval sheet

This is to approve that the dissertation presented by **Bardes Bahaa Abd El-Hakim Hassan**, to Cairo University for the Doctor of Philosophy Degree of Veterinary Science Pathology (General, Special and Postmortem) has been approved in 20/3/2016 by the examining committee.

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Abstract

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Feline mammary carcinoma (FMC) is similar to human breast cancer (HBC) in the late age of onset, incidence, histopathologic features, biologic behavior, and pattern of metastasis. There is a lack of knowledge about the existence and prognostic value of lymphangiogenesis in FMC. Therefore, FMC has been proposed as a relevant model for aggressive HBC. The goals of this study were to **1)** develop a nude mouse model of FMC growth and metastasis; **2)** measure the expression of genes responsible for lymphangiogenesis, angiogenesis, tumor progression and lymph node metastasis using FMC tissues, mouse xenografts and cell lines; and **3)** design an immunohistochemical protocol to differentiate between blood and lymphatic vessels in FMC and to compare blood and lymphatic vessel invasion detected by hematoxylin and eosin (H&E) versus that detected by immunohistochemistry (IHC). Two primary FMC tissues were injected subcutaneously and six FMC cell lines were injected into 3 sites (subcutaneous, intratibial and intracardiac) in nude mice. Tumors and metastases were monitored using bioluminescent imaging and characterized by gross necropsy, radiology and histopathology. Molecular characterization of invasion and metastasis genes in FMC was conducted using qRT-PCR in six primary FMC tissues, two subcutaneous FMC xenografts and six FMC cell lines. A survey study was done on 42 specimens from cats diagnosed with mammary cancer were stained with H&E and classified based on histopathological examination. Eighteen specimens out of 42, characterized by vascular and/or lymphatic invasion, were selected and evaluated by IHC using antibody against prospero-related homeobox domain 1 (PROX1) as lymphatic endothelial marker. The histologic appearance of the subcutaneous xenografts resembled the primary tumors. No metastasis was evident following subcutaneous injection of both tumor tissues and cell lines while lung, brain, liver, kidney and bone metastases were confirmed following intratibial and intracardiac injection of FMC cell lines. Fifteen genes were differentially expressed in the FMC tissue and cell lines and the highly expressed genes in all the samples were PDGFA, PDGFB, PDGFC, FGF2, EGFR, ERBB2, ERBB3, VEGFD, VEGFR3 and MYOF. Finally, PROX1 immunostaining was present in the nucleus of the peritratumoral lymphatic endothelial cells. This investigation demonstrated the usefulness of nude mouse models of experimental FMC and identified molecular targets of FMC progression and metastasis.

Keywords:
mammary cancer, metastasis, lung, brain, bone, angiogenesis, lymphangiogenesis, immunohistochemistry, cat

Dedication

To

My mother, my father

My sister, my brothers, my dear husband and my sons

My close colleagues, lab mates and friends

I thank all of you for your efforts with me and I hope to give all of you pleasure as you give me.

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