

# STUDY OF THE EFFECTS OF ANGIOGENESIS SUPPRESSION ON THE GROWTH OF NMU-INDUCED BREAST CARCINOMA

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## INTRODUCTION

Breast cancer is the most common cancer among women and remained as the main killer worldwide notably in Malaysia [1,2]. The growth of breast cancer is extensively supported by the formation of new vascular network out of pre-existing vessels known as angiogenesis. It is crucial for sustainable tumour growth and metastasis [3-5]. Currently, increasing studies are focusing on targeting signal transduction pathways involved in tumour cell proliferation, invasion, or tumour angiogenesis [6]. Mass studies suggested that Rapamycin, formerly known as bacterial macrolide with antifungal and immunosuppressant activities, appears to have properties of tumour angiogenesis suppression [7]. In contrast, PF4, a member of the C-X-C chemokine family, is known as angiogenesis inhibitor [8]. Thus, the current study was carried out to determine the angiogenesis inhibitory effects of Rapamycin and/or Platelet Factor 4 (PF4) on the growth of N-nitroso-N-methylurea (NMU)-induced breast carcinoma model.

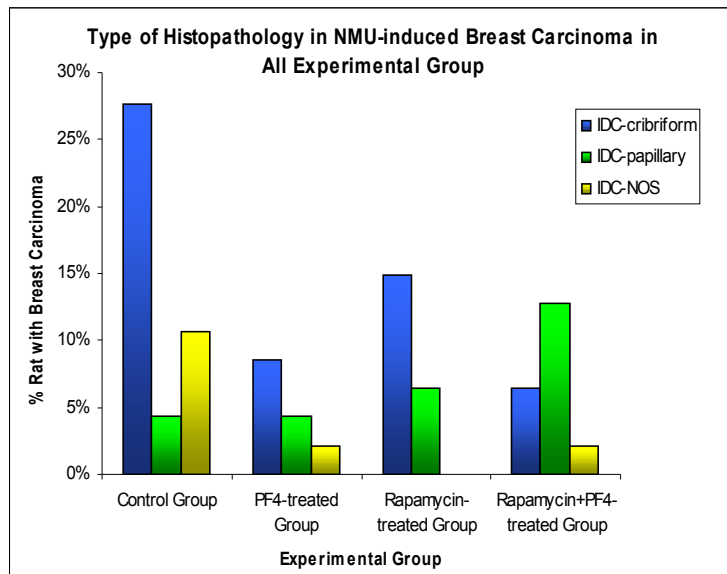
## MATERIALS AND METHODS

55 Female Sprague Dawley rats aged 21-day-old were administered intraperitoneally with NMU at a dose of 70 mg/kg body weight [9]. The animals were randomly assigned to 4 treatment groups including Group 1 (Untreated-Control), Groups 2 (Rapamycin-treated), Group 3 (PF4-treated) and Groups 4 (Combination-treated). The entire palpable tumours were excised when the tumour size reached the diameters of 7.5±0.5 mm, 11.5±0.5 mm, 17.5±0.5 mm and 20.5±0.5 mm for untreated-control group, and 7.5±0.5 mm and 11.5±0.5 mm for treated-groups. The tumours were then subjected to histopathological and immunohistochemical assessment.

## RESULTS

There is a significant association between aggressiveness of lesion and physical morphology of tumour ( $p < 0.05$ ). The histopathological pattern showed that mammary tumours developed at size 11.5 mm and less was less aggressive subtype whilst tumour size 17.5mm and beyond showed transformation to more aggressive subtype. The less aggressive subtype was histologically classified as Cribriform carcinoma while the more aggressive subtypes were histologically classified as Infiltrating Ductal Carcinoma-Not Otherwise Specified (IDC-NOS) and Papillary carcinoma. Rapamycin treatment significantly inhibited mammary

tumour progression ( $p < 0.05$ ). In contrast PF4 treatment showed markedly slowed but did not abrogate tumour growth. Meanwhile combination treatment showed tumour mass regressed and remained small, however with no improvement in total histological patterns. To further demonstrate the roles of angiogenesis in the invasiveness of tumour progression as well as tumour inhibition, immunohistochemical findings showed that untreated NMU-induced mammary tumours did undergo tumour angiogenesis notably via VEGF/VEGFR-2 signalling cascade which subsequently induced NO production through endogenous eNOS and activation of PKC $\alpha$  in tumour cells. Meanwhile, all treatment groups demonstrated that tumour inhibitions elicited through VEGF/VEGFR2 signalling pathway.



**Figure 1.** Histopathological type of NMU-induced breast carcinoma in all experimental cohorts.

Taken together, it showed that by inhibiting tumour VEGF signalling would normalise the tumour vasculature, and may shift tumours from an aggressive mammary tumour phenotype to less aggressive and perhaps to a more benign if intervention period is prolonged. Therefore, we postulated that suppression of VEGF via VEGFR-2 in Rapamycin-treated cohort resulted in attenuation of both tumour growth and angiogenesis, whereas PF4-treated cohort showed that incomplete regression of both tumour growth and angiogenesis suggested that NMU-induced mammary carcinoma secreted VEGF-lacking heparin binding. Thus PF4 is not the best regimen for anti-VEGF therapy as PF4 selectively inhibits VEGF-dependent heparin binding only. Hence, our studies suggest that Rapamycin treatment potentiated to be a novel antiangiogenesis therapy for breast cancer regimen especially when considering the fact that tumour growth is a crucial problem in advanced stages of breast cancer. In addition Rapamycin treatment exhibit non-cytotoxic effect on both the tumour cells and also host tissue compared to conventional chemotherapy.

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