EXPRESSION DISTRIBUTION OF CANCER STEM CELL, EPITHELIAL TO MESENCHYMAL TRANSITION AND TELOMERASE ACTIVITY IN BREAST CANCER, AND THEIR ASSOCIATION WITH CLINICOPATHOLOGICAL CHARACTERISTIC

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BORANG PENGESHAN STATUS TESIS

Judul: THE NARATIVE VALUE OF RED SEAWEED, *Kappaphycus alvarezil* MEAL IN FORMULATED FEED OF JUVENILE ASIAN SEABASS, *Lates calcarifer*

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20 October 2014

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ABSTRACT

Three novel concepts have been emerged in breast cancer biology: the role of cancer stem cells (CSC) in tumor initiation, and the involvement of an epithelial to mesenchymal transition (EMT) in the invasiveness and metastasis of cancer cells, along with the telomerase role in keeping the CSC immortal, and avoiding senescence. CSC are a small subpopulation of cells within tumors that initiate the tumor, telomerase is the intracellular enzyme responsible for the elongation of chromosomal telomer during each cell division, while EMT is the loss of epithelial differentiation and gained the mesenchymal phenotype.

The objective of this study is to analyse and determine the prevalence, and prognosticimportance of CSC, Telomerase activity, and epithelial to mesenchymal transition, in primary and metastatic breast carcinoma, and associationbetween each other.

A total of 167 surgically resected primary invasive breast carcinomas, and 63 metastatic lymph node lesionwere analysed forimmunohistochemical localization of the CD44+CD24-^{low}breast CSC markers, and EMT markers,vimentin and E-cadherin, by double staining IHC technique, as well as telomerase activity in formaline fixed paraffin embedded tissue, the results was validated by double staining immunoflourecent and flow cytometry techniques.

The results showed CSC with CD44+CD24-^{low} phenotype was significantly increased in node-positive tumours ($p<0.0001$), and in high grade tumors ($p<0.0001$), so CSC is independent, negative prognostic factor, its presence indicate poor prognosis, there was considerable high incidence of these cells in metastatic lymph node lesion compared to primary tumor (p-0.000), CD44+CD24-^{low} phenotypic cells was more prevalent and in significant number in ductal carcinoma insitu (DCIS) comparing to its invasive counterpart (p- 0.001). There was no significant correlation observed in between telomerase activity and clinicopathologicalbreast cancer parameters,but there was considerable high incidence of telomerase expression in metastatic lymph node lesion. EMT was more

expressed in special subtypes of invasive carcinoma comparing to IDC (NOS). The incidence of EMT was more in triple negative tumor. EMT expression was more prevalence in DCIS lesion relative to its invasive component (p-value 0.000),and there was considerable high number of tumor cells with EMT expression in metastatic lymph node lesion (p-0.001).The occurrence of EMT phenomena was usually accompanied by the co-existence of CSC of CD44+CD24-^{low} phenotype. There was no association between the existence of CSC and detection of telomerase activity in tumor cells.

Increase number of both CSC of CD44+CD24-^{low}phenotype and cells underwent EMT in DCIS lesion might be an initial step in the stromal invasion and propagation of breast cancer, and induction of EMT in the breast tumor associated with high prevalence of CSC, promoting tumorinvasiveness and metastasis.
EMT occurrence is always co-existence with CSC subsistence, suggesting that EMT phenotype induced by different factors are rich sources for CSC, which raise the possibility of biological similarities between CSC, and EMT-phenotypic cells.

Finally, we can conclude that the currently used detection methods for breast CSC and EMT are not enough to identify all subtypes of these tumor cell. The clinical relevance on prognosis and therapy response has to be further evaluated in a prospective trial.

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AACR	American Association for Cancer Research
AEC	3-amino-9-ethyl carbazole
AJCC	American joint committee on cancer
AMCA	aminomethylcoumarin acetate
BSA	Bovine serum albumin
CSC	Cancer stem cell
DAB	3,3′-diaminobenzidine
DAPI	diamidino-2-phenylindole
DCIS	Ductal carcinoma insitu
DFS	Disease-free survival
DPX	Distrene 80, polystyrene, and Xylene
DW	Distal water
EDTA	Tris-ethylenediamine tetra acetic acid
ER	Estrogen receptor
EMT	Epithelial mesenchymal transition
FBS	Fetal bovine serum
FFPES	formalin-fixed paraffin-embedded tissue sections
FITC	FlouresceinIsoThioCyanate
FISH	FlourescentinsituHybridyzation
FC	Flow cytometry
HMEC	Human mammary epithelial cell
HMW	high molecular weight
HIER	heat-induced epitope retrieval
HRP	Horse-radish peroxidase
hTERT	human telomerase reverse transcriptase
hTR	human telomerase RNA
IARC	International Agency for Research on Cancer
IDC	Invasive Ductal carcinoma
IHC	Immunohistochemistry
IF	Immunoflourescent
IDC	Invasive ductal carcinoma

ILC	Invasive Lobular carcinoma
LCIS	Lobular carcinoma insitu
LN	Lobular Neoplasia
NET	NeuroendocrineTumors
NOS	not-otherwise-specified
NOD/SCID	non-obese diabetic mice with severe combined immunodeficiency disease
MET	Mesenchymal to epithelial transition
MC	Medullary Carcinoma
OS	overall survival
PAS	periodic acid-Schiff
PBS	phosphate-buffered saline
PR	Progestrone receptor
RT-PCR	Real time-polymerase chain reaction
SCS	single cell suspension
TW	Tap water
TBS	Tris-Buffered Saline
TDLU	Terminal Duct Lobular Unit
TRAP	Telemetric Repeat Amplification Protocol
UICC	International Union Against Cancer
WHO	World health organization

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CHAPTER 1

INTRODUCTION

1.1 Research background

Breast carcinoma is the most common malignant tumor and the leading cause of carcinoma death in women, with more than 1 000 000 cases occurring worldwide annually. The Malaysian National Cancer Registry 2006 reported that there were 3,525 female breast cancer cases in Malaysia and this made it the most commonly diagnosed cancer in women (29.9 % of all new cancers) (Malaysian MOH and Academy of Medicine Malaysia, 2010).

Human breast cancer is a truly complex disease with a large inter-tumoral and intra-tumoral heterogeneity resulting in highly variable clinical behavior and response to therapy. The maintenance of the heterogeneity of cells within a tumor is not fully understood. Possibly, every cell within a tumor may have a capacity to proliferate and form new tumors, although the likelihood for each cell is very low. Alternatively, only a small subset of cells with distinct characteristics has the capacity to maintain tumor growth (Gabriella, H *et al.,* 2008), called cancer stem cells (CSC), which are capable of both tumor initiation and sustaining tumor growth.

Three novel concepts have emerged in breast cancer biology: the role of cancer stem cells in tumor initiation, and the involvement of an epithelial to mesenchymal transition (EMT) in the metastatic dissemination of cancer cells, along with the Telomerase role in keeping the CSC immortal, and avoiding senescence.
The clinicopathological significance of these three novel concepts in primary and metastatic breast carcinoma, and correlation among each other are the leading purpose of this research.

1.1.1 Cancer stem cell

The first novel issue is the CSC concept, these cells possess distinct immunological markers, they have CD44+/CD24-/^{low} phenotype, they has been demonstrated by Al-Hajj *et al.* (2003) to have tumor-initiating properties in breast cancer. This tumorigenic phenotype has been associated with stem cell-like characteristics (Ponti, D *et al.,* 2005) , with enhanced invasive properties (Sheridan *et al.,* 2006), and radiation resistance (Phillips *et al.,* 2006).

The concept of cancer stem cells has led to new hypotheses about tumor growth. Cancer stem cells share similar properties with normal stem cells in terms of their capacity for self-renewal. They can self renew, cause tumorigenesis, recurrence, and metastasis. Moreover, they can divide asymmetrically to generate differentiated cancer cells within the population of cancer cells, cancer stem cells are the ones that can form new tumors, and their asymmetric division contributes to the heterogeneity (Wendy *et al.,* 2008).

The CSC hypothesis posit that this minority of cells population can fuel and drive tumor growth and remain in patients after conventional chemotherapy which eradicate the rapidly growing non tumorigenic cell which constitute the major bulk of the tumor, then it is unlikely to be curative and relapses would be expected .The hypothesis predicts that effective tumor eradication will require obtaining agents that can target cancer stem cells while sparing normal stem cells. This explains why the cancer stem cell hypothesis is at the center of a rapidly evolving field that may play a pivotal role in changing how basic cancer researchers, clinical investigators, physicians, and cancer patients view cancer (Michael *et al.,* 2006).

It is widely accepted that CSC originated from pluri-potent normal stem cell rather than from differentiated progenitor cells. Two basic arguments underlie the hypothesis that cancer stem cells originate from normal tissue stem cells. First, as tumor development is believed to result from the sequential and progressive accumulation of genetic abnormalities, adult stem cells appear to be ideal initial targets for malignant transformation due to their long life spans. Second, CSCs