THE RELATIONSHIP BETWEEN

MAST CELL DENSITY AND MICROVESSEL DENSITY

WITH GRADING OF BREAST CARCINOMA

BY

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LIST OF ABBREVIATIONS

CD	Cluster of differentiation
CSC	Cancer stem cell
DNA	Deoxyribonuleic acid
ER	Estrogen receptor
FGF	Fibroblast growth factor
HER2	Human epithelial growth factor 2
LOH	Loss of heterozygosity
MMP	Matrix metalloprotease
NK	Natural killer
PR	Progesterone receptor
SCF	Stromal colony factor
TNF	Tumour necrosis factor
TSP1	Thrombospondin 1
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

ABSTRACT

The relationship between mast cell density and microvessel density with grading of breast carcinoma

There is increasing evidence that indicates the essential role of tumour microenvironment in relation to tumour initiation and progression. The need to further explore this role is crucial as it can provide important prognostic and predictive information on tumour as well as offering potential targets for therapeutic attack.

In this study, we aimed to analyze the relationship between mast cell density and microvessel density with grading of breast carcinoma and the correlation between these two variables. In addition, we also analyzed the relationship between MCD and MVD with other clinicopathological parameters.

95 cases of breast carcinoma, diagnosed within 2009 to 2011 in Hospital Raja Permaisuri Bainun, Ipoh, were reviewed. We observed significant association between mast cell density with breast carcinoma grade ($p=0.04$), with higher count seen in low grade tumour. However, there was no significant association seen with microvessel density with grading of breast carcinoma ($p=0.25$). Both mast cell density and microvessel density did not show any statistically significant association. Among the clinicopathological parameters, it was observed that high mast cell density was seen in cases negative for lymph node invasion ($p=0.01$), whereas high microvessel density was noted in cases positive for vascular invasion ($p=0.03$). Other clinicopathological parameters did not show statistically significant association.

As a conclusion, higher mast cell density was seen in low grade tumour and in cases without lymph nodes metastasis, as observed in other studies. However, the finding for microvessel density did not concur with other similar studies.

ABSTRAK

Terdapat semakin banyak bukti yang menunjukkan peranan sel dalam tisu persekitaran kanser yang menyumbang kepada perkembangan sesuatu kanser. Oleh itu, kajian dalam konteks ini amat penting memandangkan ia dapat memberi maklumat prognostifikasi serta berperanan sebagai satu rawatan alternatif.

Kami menkaji hubungan di antara 'mast cell density' dan 'microvessel density' dengan gred tumor serta kaitan antara keduanya. Selain itu,kami juga menkaji hubungan antara dua parameter di atas dengan faktor-faktor klinikalpatologikal seperti umur pesakit, saiz tumor dan lain-lain.

95 kes barah payu dara yang telah didiagnosa dalam tahun 2009 ke 2011 di Hospital Raja Permaisuri Bainun, Ipoh telah dianalisa. Kami mendapati terdapat hubungan yang signifikan antara 'mast cell density' dengan gred tumor (p=0.04), di mana terdapat lebih tinggi 'mast cell density' dalam kes yang rendah gred tumor. Walau bagaimanapun, tiada hubungan yang signifikan di antara 'microvessel density' dan gred tumor (p=0.25). 'Mast cell density' menunjukkan hubungan yang signifikan dengan penglibatan tumor pada limfa (p=0.01), manakala 'microvessel density' menunjukkan hubungan yang signifikan dengan penglibatan tumor pada salur darah (p=0.03).

Kesimpulannya, keputusan yang menunjukkan hubungan signifikan antara 'mast cell density' dengan gred tumor, di mana lebih tinggi 'mast cell density' dilihat dalam kes

yang rendah gred tumor dan kes yang negatif untuk penglibatan tumor pada limfa, adalah seperti yang dilihat dalam kajian-kajian lain sebelum ini. Walau bagaimana pun, keputusan untuk 'microvessel density' yang tidak menunjukkan hubungan signifikan dengan gred tumor dan factor-faktor klinikopatological adalah bercanggah dengan keputusan kajian lain.

CHAPTER 1:

INTRODUCTION

1.0 INTRODUCTION

Breast carcinoma is the most common carcinoma in women in most countries. It accounts for 22% of all female cancer (Fattaneh, et al., 2002). In 2003, the World Health Organisation predicted that more than 1.2 million women were diagnosed with breast cancer, which represents a significant global disease burden.

In Malaysia, breast cancer is the most common female cancer and the commonest cause of death due to cancer for Malaysian women. In National Cancer Registry 2003, there were 3738 female breast cancer cases reported with the overall age standardized risk of 46.2 per 100,000 population.

Since breast cancer causes significant morbidity and mortality within a significant number of women worldwide, there were impressive advances have been made within the past 50 years as an effort to prevent, treat and cure breast cancer. Major achievement includes early detection by development of mammography, the availability of antiestrogenic compounds for inhibition of disease progression, advances in chemotherapy for primary treatment and as adjuvant therapy and also introduction of sentinel lymph node mapping for staging. This has lead to a better prognosis. All these would not have been feasible without the abundance and extensive research done for breast cancer.

Recently, much interest is seen in tumour microenvironment. There are increasing evidence which indicates tumour-stromal cell interaction plays an important role for tumour initiation and progression (Werb, 2010). Thus, it would be interesting and beneficial to study this area in one of the commonest cancer in our population. Although there are a number of studies already conducted in this matter, none was conducted in the Asian population. As a few studies have shown that breast cancer behaved differently in different population (Edward, et al., 1998) and the trends in breast cancer differ by race and ethnicity (Ghafoor, et al., 2003) it would be imperative to understand the diversity of tumour microenvironment in different population, particularly in relation of instituting immunological therapy.

CHAPTER 2:
LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 Incidence and Epidemiology

Breast carcinoma is the most common cancer in women, where it accounts for almost a quarter of all female malignancies (Fattaneh, et al., 2002). According to WHO 2003, it contributes to 26% of all cancer rate in developed countries, such as Europe, North America and Australia in which 6% of women are affected by invasive breast carcinoma before reaching 75 years. However, the probability for developing breast cancer is much less in developing countries within Asia and sub-Saharan Africa.

In Malaysia, breast cancer is also the commonest cancer within our female population, accounted for 31% of all newly diagnosed cancer in women (National Cancer Registry, 2003). There were 3738 new cases diagnosed in 2003 with ASR of 46.2 per 100,000 population. This translates to 1 in 20 Malaysian women being at risk of developing this cancer within their lifetime.

The prevalent age group was those within 50-59 years old within all ethnics. The rates dropped in the older age group. Among the ethnic group in Malaysia, the Chinese show the highest incidence with an ASR of 59.7 per 100,000 whereas the Malays had the lowest incidence with an ASR of 33.9 per 100,000 population.

2.2 **The normal breast**

The breasts in humans are modified sweat glands which rest on the pectoralis muscle separated by a fascia. They are composed of specialized epithelium and stroma, each can be involved in either benign or malignant conditions.

The breast consists of complex branching structure composed of six to ten major ductal systems that divides into multiple terminal duct-lobular units (TDLU). The terminal duct in adult women forms a grape like clusters of small acini to form lobules. They provide the secretory function of the gland. The secretion would drain into subsegmental duct, then segmental duct and followed by lactiferous duct that finally lead to the nipple.

The breast and lobules are lined by 2 cell types. The inner cuboidal epithelium has absorptive and secretory function whereas the outer contractile myoepithelial layer contain myofilament which assist in milk ejection and provide structural support for the lobules. All the glands lie on a continuous basement membrane. There are also 2 types of breast stroma. There are dense fibrous connective tissue and fat within the interlobular stroma, whereas the intralobular stroma show presence of specialized hormonally responsive fibroblast-like cells plus some scattered lymphocytes.

The nipple shows its own characteristic appearance (Rosai, et al., 2010). It is lined by stratified squamous epithelium and contains multiple collecting ducts, sebaceous glands and hair follicles. The difference in between the nipple and its surrounding areolar from other skin epidermis is due to the presence higher number of melanocyte and scattered basally located clear cell known as Toker's cell.

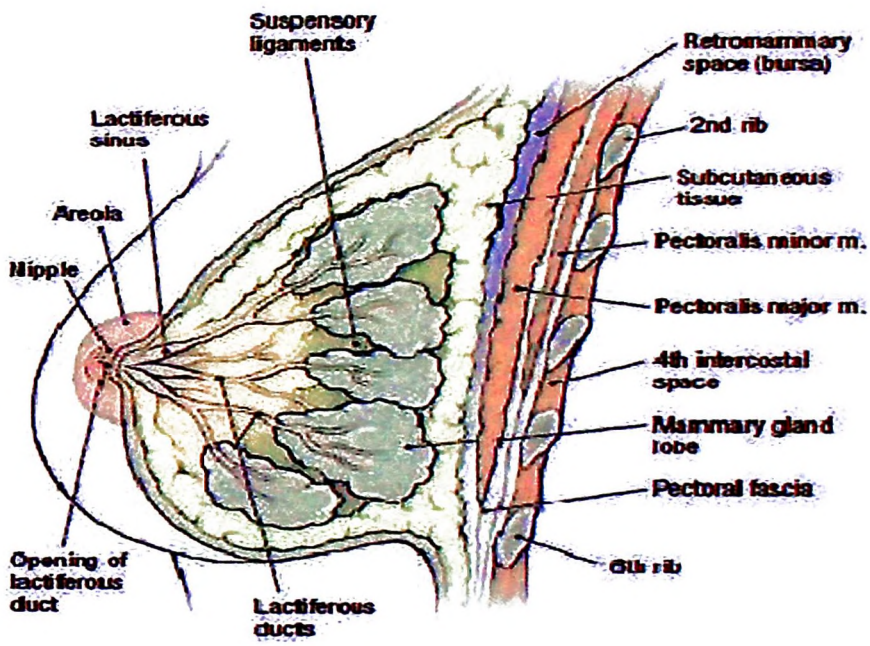

Figure 2.1 : Anatomy of breast (Adapted from Clinical Anatomy for Medical Student 6th Edition by Richard R. Snell, 2000)

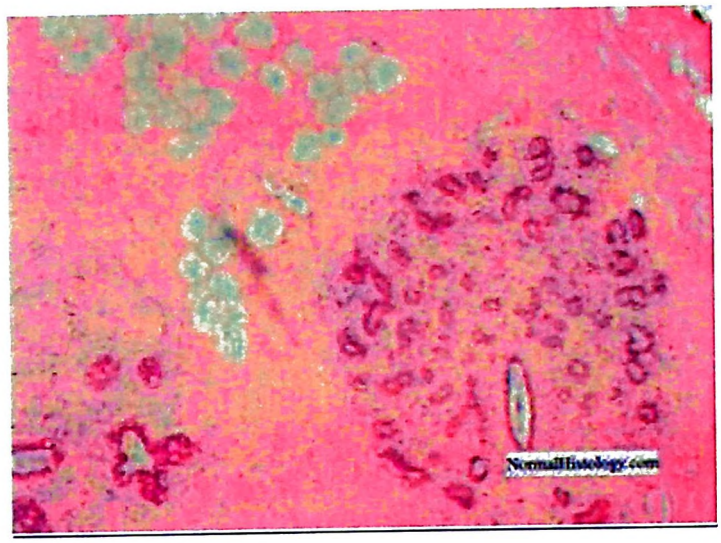

Figure 2.2 : Histology of normal adult breast (Adapted from Histology for Pathologist 3rd Edition by Stacey E. Mills, 2007)

The breast shows dependence on hormonal influence for its normal activity, which is manifested by structural and functional changes seen throughout a women's life. There are subtle changes that occur during normal menstrual cycle, however, major changes occur during puberty, pregnancy, lactation and menopause (Mills, 2007). However, hormonal disturbances may have some influence in various breast disorders, in particular breast carcinoma.

2.3 **Etiology and pathogenesis**

The etiology of breast cancer is multifactorial. It is most likely constitutes an interplay of genetic factors and environmental factors (reproductive factors, hormonal imbalance, nutrition, alcohol, physical activity and ionizing radiation).

However, genetic and hormonal exposure remains the major risk factors for development of breast cancer. Thus, breast carcinomas can be divided into hereditary cases with associated germline mutations and sporadic cases that are probably related to hormonal exposure (Vinay Kumar, et al., 2010).

Hereditary breast cancer

In approximately 12% of breast cancers, the primary cause is inheritance of a susceptibility gene. This is highly probable in the presence of multiple affected first-degree relatives, diagnosed before menopause and/or have multiple cancers or there are family members with specific cancers.

Mutation in BRCA1 and BRCA2 accounst for the majority of cancers attributable to single mutations and about 3% of all breast cancers (Vinay Kumar, et al., 2010). Penetrance varies from 30% to 90% depending on the specific mutations present.

Other groups of known susceptibility genes accounts for fewer than 10% of hereditary breast carcinomas. This includes Li-Fraumeni syndrome (due to germline mutation in p53) and Li-Fraumeni variant syndrome (due to germline mutation in CHEK2) account for 8% of breast carcinoma caused by single gene. Other tumour suppressor genes such as PTEN (Cowden syndrome), ATM (ataxia telangiectasia) and LKBI/STK11 (Peutz-Jeghers syndrome) show mutation in less than 1% of all breast cancers (Vinay Kumar, et al., 2010).

Thus, it is shown that only about one quarter of familial breast cancer is caused by known high-risk breast cancer genes. Therefore, it is likely that the remaining familial cancers are caused by multiple genes with weak effects. The presence of a number of candidate genes associated with risk of developing breast cancer had been identified in a number of genome-wide association studies (GWAS). This includes fibroblast growth factor receptor-2 (FGFR2).

The major susceptibility genes for breast cancer are tumour suppressors that have normal roles in DNA repair, cell cycle control and the regulation of apoptosis in many tissues. Except for p53, mutation in genes implicated in hereditary breast cancer are uncommon in sporadic breast cancer (Robert J. Kurman, et al., 2002). However, decreased expressions of BRCA1 and CHEK2 is

common in sporadic cancers, particularly those that are triple-negative or poorly differentiated and basal-like cancer, which comprise a large subset of triple-negative group, have gene expression profile that bears a striking resemblance to hereditary cancer arising in BRCA1 carriers (Vinay Kumar, et al., 2010). Based on these observations, it is suspected that the pathway that these genes participate in a frequently disrupted in sporadic cancer through currently unknown mechanism.

Sporadic breast cancer

The major risk factors for sporadic breast cancer are related to hormone exposure: gender, age at menarche and menopause, reproductive history, breast feeding and exogenous estrogen. Thus, it is observed that majority of sporadic breast cancer occurs in postmenopausal women and are ER positive.

As a result of hormonal exposure, the number of potential target cells is increased by stimulation of breast growth during puberty, menstrual cycle and pregnancy (Robert J. Kurman, et al., 2002). The effect of hormonal exposure also increases the cell proliferation, causing additional risk for DNA damage. Apart from endogenous estrogen, exogenous source such as from hormone replacement therapy is also implicated (Fattaneh, et al., 2002). Once premalignant or

malignant cells are present, hormones can stimulate their growth as well as the growth of normal epithelial and stromal cells that may aid in tumour progression.

Estrogen may also play a more direct role in carcinogenesis (Robert J. Kurman, et al., 2002). Its metabolite can cause mutation or produce free-radicals in cells. This is proposed by a few variants of genes involved in estrogen synthesis and metabolism which is related to the increase risk of breast cancer. Such variants would also be analogous to cytochrome P-450 allele that alter the metabolism of tamoxifen (Vinay Kumar, et al., 2010).

2.4 **Overview of carcinogenesis and tumour progression**

The diverse histologic appearances of carcinomas and precursor lesions are the outward manifestations of the complex genetic changes that drives carcinogenesis. One model of carcinogenesis postulates that a normal cell must acquire several new capabilities to become malignant (Vinay Kumar, et al., 2010). Each may be achieved by a change in the activity of one of many different genes that regulate common cellular activities.

Population of cells that harbour some, but not all, of the genetic changes that are required for carcinogenesis give rise to morphologically recognizable

breast lesions that are associated with an increase risk of progression to cancer (Vinay Kumar, et al., 2010). The earliest such alterations are proliferative changes, which may stem from the loss of growth inhibiting signals or decreased apoptosis. For example, most early lesions (such as atypical ductal hyperplasia and atypical lobular hyperplasia) show increase expression of hormone receptors and abnormal regulation of proliferation. LOH is rarely detected in typical proliferative change but becomes more frequent in atypical hyperplasias and is almost always present in carcinoma in situ (Fattaneh, et al., 2002). Profound DNA instability in the form of aneuploidy, which manifest morphologically as nuclear enlargement, irregularity and hyperchromasia, is observed in in high grade DCIS and some invasive carcinomas (Vinay Kumar, et al., 2010). At some point during tumour progression the malignant clone also become immortalized and acquire the ability to drive neo-angiogenesis (Robert J. Kurman, et al., 2002). The morphology and biological features of carcinomas are usually established at the in-situ stage, since in the majority of cases the in-situ lesion closely resembles the accompanying invasive carcinoma.

The cell of origin of breast cancer is of interest, since this has important implication for etiology and treatment (Britta Weigelt, et al 2010). The cancer "stem cell hypothesis" proposes that malignant changes occur in a stem cell population that has unique properties distinguishing them from more differentiated cells (Foulkes, et al 2010). Although the majority of tumour cells

would consist of non stem cell progeny, only the malignant stem cell would contribute to tumour progression or recurrence.

The most likely cell-type of origin for the majority of carcinoma is the ER-expressing luminal cell, since the majority of cancer are ER positive and precursor lesions, such as atypical hyperplasias, are most similar to this type of cell (Britta Weigelt, et al 2010). ER negative carcinoma may arise from ER negative myoepithelial cells. This would explains why many proteins found in myoepithelial cells are shared by the triple negative or basal-like cancers (Foulkes, et al 2010). An alternative possibility is an origin from an ER positive precursor that loses ER expression. The precursor lesion of ER negative tumour is unknown.

The final step of carcinogenesis, the transition of carcinoma-in-situ to invasive carcinoma, is the most important but unfortunately the least understood (Vinay Kumar, et al., 2010). Genetic markers specific for invasive carcinomas have been difficult to identify. It is important to remember that the structure and function of the normal breast depend on a complex interplay between luminal cells, myoepithelial cells, and stromal cells (Fattaneh, et al., 2002). The same molecular events that allow for the normal formation new ductal branch point and lobules during puberty and pregnancy-abrogation of the basement membrane,

increase proliferation, escape from growth inhibition, angiogenesis and invasion of stroma-may be recapitulated during carcinogenesis (Vinay Kumar, et al., 2010).

As a summary, there are multiple pathways which lead to the development of breast cancer. As researchers, we should keep in mind that breast carcinoma is a heterogenous group of cancer, which displays a variation in morphology, molecular characteristic, clinical features and response to treatment (Britta Weigelt, et al., 2010). This can be manifested by the existence of several ways to classify breast carcinoma.

2.5 **Classification of breast carcinoma**

The need to classify breast carcinoma is the same as in any other diseases, in which to guide the direction of treatment and provide prognosis based on the type of breast carcinoma diagnosed.

The traditional way to classify breast carcinoma is by its morphological features. In WHO 2003, it is categorized into ductal carcinoma, lobular carcinoma, tubular carcinoma, invasive cribiform carcinoma, medullary carcinoma, mucin producing carcinoma, neuroendocrine carcinoma, invasive

micropapillary carcinoma, metaplastic carcinoma and other rare forms of carcinomas. Some histological type, such as mucinous, adenoid cystic, tubular and invasive cribiform carcinoma show favourable clinical outcome (Fattaneh, et al., 2002). However, the lymph node status and the number of lymph nodes with metastasis provide the most important single prognostic factor (Fitzgibbons, et al., 2000). Tumour size, measured by the size of the invasive component is also another significant prognostic factor (Abner, et al 1998). Apart from these two factors, the histological grade (Pereira, et al., 1995), tumour cell proliferation (Fitzgibbons, et al., 2000) and presence of lymphatic and blood vessel invasion (Lee, et al., 1997; Leitner, et al., 1995) are associated with unfavourable outcome with higher histological grade, high proliferative index and positive of invasion. Other predictive factors with less conclusive result are presence of tumour necrosis (Gilchrist, et al., 1993) and extent of ductal carcinoma in situ (DCIS) component (SJ S, et al., 1997). Interestingly, morphological appearance of tumour stroma also has been reported with conflicting result, with some studies reporting no prognostic significance, good prognosis or adverse prognosis (Fattaneh, et al., 2002). The Nottingham Prognostic Index attempts to integrate the histological prognostic factors by stratifying patients with breast carcinoma into good, moderate and poor prognostic groups with correlate with annual mortality rates of 3%, 7% and 30% respectively (Galea et al, et al., 1992).

Although the conventional method of classification had so far stood the test of time, this had been recently challenged by gene expression profiling. This

method, which uses the cDNA microarray to discover the gene expression pattern, managed to classify breast carcinoma into 5 subtypes, each represented by a distinct set of genes. (Britta Weigelt, et al., 2010). The 5 subypes are basal-like, luminal A, luminal B, human epithelial growth factor receptor 2 (HER2) overexpressing and normal breast-like. A study by Sorlie et al reported that the 5 subtypes show significant differences in overall and relapse-free survival within each subtype, with basal-like subtype having the shortest survival. This result was also seen in a few other studies (Sorlie, et al., 2003). Another example can be seen in luminal B subtype, which may be a representative of a class of ER-positive tumour with poor prognosis, possibly not responding to tamoxifen (Britta Weigelt, et al., 2010). Despite the presence of these findings, at this stage gene expression profiling still do not fully established function in clinical application. It can be attributed to heterogenous in sample selection and numbers of tumours being analyzed in the studies conducted within this area. Furthermore, only some of these tumours show a characteristic morphology, such as basal-like breast carcinoma. Even then, there is still no general agreement on the immunophenotypic criteria of basal-like breast carcinoma.

Another frequently mentioned group of breast carcinoma is triple-negative breast carcinoma. It is a tumour characterized by lack of estrogen receptor, progesterone receptor and HER2 expressions. There is difference in defining the cut off point of ER, PR and HER2 expression among the researchers. Some accept tumours as being negative for ER or PR only if less than 1% of the cells

are positive for ER or PR expression whereas others accept the cut off point as 10%. For HER2, 2 most commonly adopted include tumours with immunohistochemical score of 0/1+ or tumours with scores of 0/1+ or 2+ that are lacking HER2 gene amplication after in situ hibridization. It is observed that this type of breast carcinoma occurred more frequently in young black and Hispanic women than in young women from other racial groups with usually high histologic grade. BRCA1 is an important breast-cancer susceptibility gene in this type of tumour. As a group, patients with triple-negative breast carcinoma have a relatively poor outcome and cannot be treated with endocrine therapies or therapies targeted to HER2 (Foulkes, et al.,2010).

A Pubmed search of medical literature failed to show any studies related to mast cell density and triple negative breast carcinoma. However, there were several articles based on studies that explore the relationship between angiogenesis and triple negative breast carcinoma. A study by Liu T.J., et al demonstrated that CD133 expression was highest in triple negative breast carcinoma specimen. By using triple negative breast carcinoma cell line, MDA-MB-231 cells, they discovered that these cells produced a range of colony morphologies, in which the presence of holoclone cells which showed higher self-renew potential and might harbors cancer stem cell subpopulation. Strikingly, the holoclone cells displayed CD133 expression and formed vasculogenic mimicry. Vasculogenic mimicry (VM) refers to the unique capability of aggressive tumour cells to mimic the pattern of embryonic vasculogenic networks (Liu T.J., et al,

2012). In addition, holoclone cells acquired endothelial cell marker vascular endothelial-cadherin expression and upregulated VM mediators, matrix metalloproteinase MMP-2 and MMP-9 expression. Thus, they concluded that the subpopulation with holoclone morphology, CD133(+) phenotype and CSC characteristics might have the capacity of transdifferentiation and contributed to VM in triple negative breast carcinoma.

Another study by Gubin MM et al explored the role of RNA binding protein HuR in triple negative breast carcinoma. Previous studies had shown that elevated levels of cytoplasmic HuR directly correlates with increased invasiveness and poor prognosis for many cancers, including breast. HuR controls the expression of multiple genes involved in angiogenesis including VEGFa, HIF1a and thrombospondin 1 (TSP1). The finding in their study showed MDA-MB-231 cells with higher levels of HuR have alteration in cell cycle kinetics and faster growth possibly by increasing in the expression of TSP1. Thus, they concluded that HuR may be regulating a cluster of genes involved in blood vessels formation, which controls tumour angiogenesis (Gubin M.M., et al, 2012).

2.6 **Grading of invasive carcinoma**

The histological grading of breast carcinoma has been incorporated into multiple, validated, prognostic algorithms to determine breast cancer therapy, such as in Nottingham Prognostic Index (Galea, et al., 1992). It demonstrated a significant association between histological grade and survival rate of patients with invasive carcinoma, that makes grading as an important prognostic factor.

Grade is an assessment of the degree of differentiation (i.e. tubule formation and nuclear pleomorphism) and proliferative activity (i.e. mitotic index) of a tumour (Ellis, et al., 1992). For assessment of tubule formation, only structures showing clear central lumina are counted. The degree of nuclear pleomorphism is compared to the regularity of nuclear size and shape of normal epithelial cells in adjacent breast tissue. Other than that, the irregularity of the nuclear outline, number and size of nucleoli should also be assessed. For calculation of mitotic count, care should be taken to include only defined mitotic figures to be included. Counting should be done within the periphery edges of the tumour within a fixed field area or by using a grid system. For each criteria, a numerical scoring system of 1-3 is used, then the three values are added to produced scores of 3-9 where the grade will be given as shown in the table below.

Point	Grade
3-5	Grade 1 - well differentiated
6-7	Grade 2 – moderately differentiated
8-9	Grade 3 – poorly differentiated

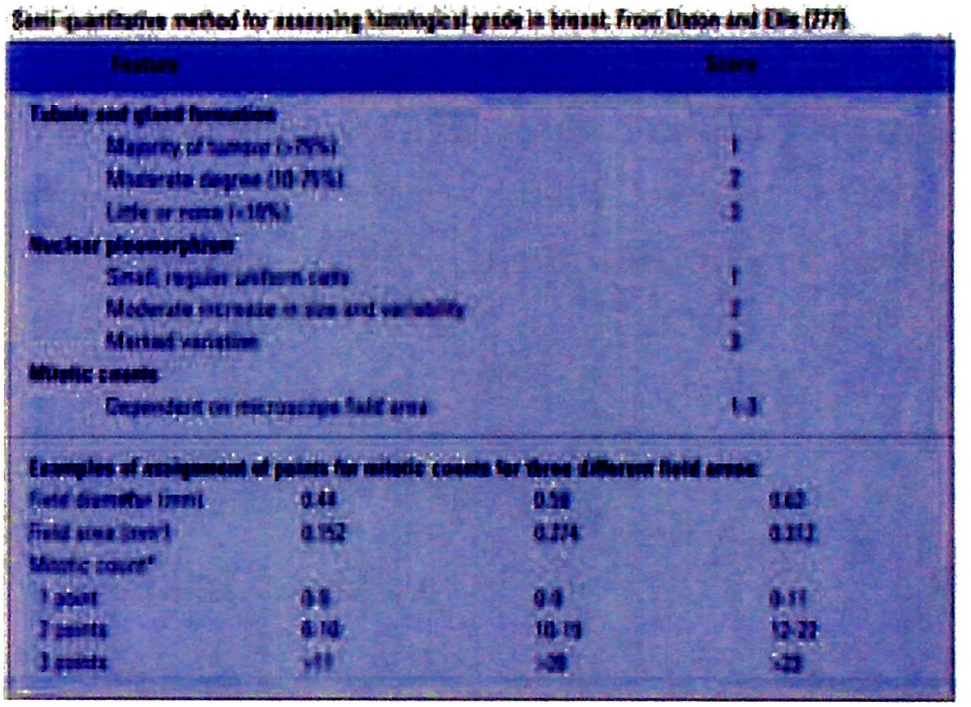

Figure 2.3 : Method of grading of invasive breast carcinoma (Adapted from WHO Classification of Tumour: Tumours of the Breast and Female Genital Organ,2002)

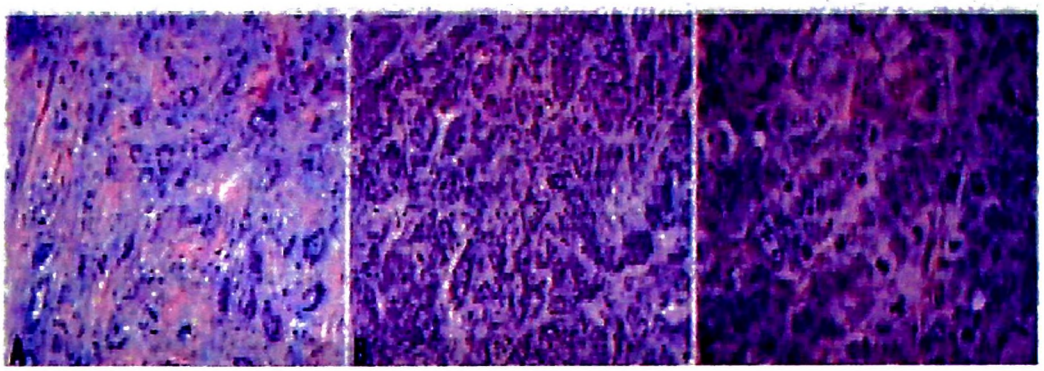

Figure 2.4 : (A) Infiltrating ductal carcinoma Grade 1 (B) Infiltrating ductal carcinoma Grade 2 (C) Infiltrating ductal carcinoma Grade 3 (Adapted from WHO Classification of Tumour: Tumours of the Breast and Female Genital Organ,2002)

2.7 **Tumour microenvironment**

The previous discussion had been on the process of carcinogenesis of breast carcinoma. However, this research project is more related to the stroma surrounding the malignant epithelial cells of the breast, so-called tumour microenvironment.

There are increasing data that indicate the tumour-stromal cell interaction provides an important role in tumour initiation and progression (Balkwill, 2001).

This is not a new concept, since it was first hypothesized by Rudolph Virchow in 1863 that the origin of cancer was at sites of chronic inflammation. This is the result of presence of chronic irritant, causing tissue damage and chronic inflammation, leading to enhanced cellular proliferation. However, at that time, the focus was only on inflammation and the roles of other stromal elements were not considered. It was Bissel et al in 1982 that highlighted the changes in tumour microenvironment that may contribute to tumour development and progression. Nowadays, the causal relationship between inflammation, innate immunity and cancer has been widely accepted. This can be seen in numerous studies conducted with regards to this matter such as in colorectal carcinoma (Gulubuva, et al., 2007), lymphoma (Molin, et al., 2002) and melanoma (Ribatti, et al., 2003), among others.

The rate of tumour growth and its progression as well as its ability to metastasize is regulated by a delicate balance between pro and anti-tumorigenic effects, produced by the tumour cells and the surrounding microenvironment. The presence of local inflammation within the tumour stroma leads to the presence of a variety of cell types, which include myeloid cells such as macrophages, mast cells, eosinophils and dendritic cells, T cells, NK cells and also endothelial precursors. There are numerous studies with similar theme in breast carcinoma (Aaltoma, et al., 1993; Ashish, et al., 2007; Gui, et al., 2005; Leek, et al., 1996). Among the cells that are investigated include macrophage (Leek, et al., 1996), mast cell (Aaltoma, et al., 1993) and stromal fibroblasts (Qu, et al., 1995).

2.8 Mast cell in tumour growth

The presence of mast cell surrounding and within many solid tumour has been noted since 1891 (Maltby, et al., 2010), with their role of either being in favour or against tumour growth. Mast cells originated from hematopoietic stem cells in the bone marrow. They exit from the marrow as committed but undifferentiated precursor, where they go in the circulation to their target organ and undergone terminal differentiation. These mature mast cells are present in most tissue with more concentration in skin, respiratory tract and gastrointestinal tract, where they act as first line of defence against pathogen. They have variable functions in different conditions such as inflammatory mediators in allergies, arthritis and asthma or tissue remodelling role in wound healing.

In tumours, mast cells infiltrations are noted at an early stage of many tumours or even at dysplastic stage. For example, mast cells infiltration is noted surrounding areas with dysplastic skin, prior to melanoma or other skin tumour progression (Ribatti, et al.,2003; C'hng, et al., 2006) and around adenomatous polyps (Gounaris, et al., 2007).

In relation to tumour growth, mast cells potential effects can be seen through angiogenesis, mast cell mediated tissue remodelling and mast cell related immune modulation (Maltby, et al., 2010).

Mast cell mediated angiogenesis

Angiogenesis is a very important component for tumour progression, as will be discussed later. In normal or non tumoural conditions, mast cell accumulation is noted surrounding blood vessels in normal homeostasis where it produces pro-angiogenic mediators such as heparin (CJ. M, et al., 1995), VEGF (Grutzkau, et al., 1998), histamine (Theoharides, et al., 1992) and TNFa (Theoharides, et al., 2004). This has lead to the suggestion that mast cells may play a role in tumour angiogenesis.

There are several studies which support this theory. According to Coussens et al., mast cells infiltration into developing tumours plays a role in triggering "angiogenic switch". This is demonstrated in their study, by using a transgenic mouse model expressing HPV 16 early region genes in basal keratinocytes that would develop tumour in first year of life, showed increase mast cells infiltration within the proximity of small blood vessels in the surrounding tumour stroma during hyperplastic stage of tumour growth (Coussens, et al., 1999). These mast cells expressed mMCP-4 and mMCP-6, where the former has angiogenic property and the latter is more involved in tissue remodelling.

Two separate studies show the role of mast cells in adenomatous polyps. In a study using APC 468 mice and transgenic mouse overexpressing B-catenin,

which are hereditary models of polyposis (Gounaris, et al., 2007), there was accumulation of CD34+ mast cell, mostly of intraepithelial mucosal type in the developing polyps, whereas when the tumour progress in invasive stage, connective tissue type of mast cell predominates. This implies a switch in mast cell subtype as the tumour progresses. Another study (Oguma, et al., 2008) shows TNFa, stimulated by mast cells has direct effect on tumour growth by activation of Wnt signalling in K19-Wnt1 transgenic mice.

Finally, another study by Nakayama et al shows the role of mast cell derived angiopoeitin 1 (Ang-1) and VEGF-A in mouse model of multiple myeloma. There was increased angiogenesis in the existence of both plasmacytoma cells and mast cells compared to when each cells were present individually. This phenomena was reduced with administration of antibodies against Ang-1 and VEGF-A.

Mast cell's role in tissue remodelling

Mast cells also produce a number of proteases which has their effect on the extracellular matrix. This effect is seen in wound healing and conditions such as asthma or autoimmune arthritis. In tumour, it has been implied that there is disruption in normal mast cell induced tissue remodelling resulting in changes to the extracellular matrix. This leads to release of some matrix-bound factors such