INVESTIGATION OF YB-1 GENE EXPRESSION BY SYBER-GREEN BASED REAL-TIME PCR IN BRAIN TUMOR

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INVESTIGATION OF YB-1 GENE EXPRESSION BY SYBER-GREEN BASED REAL-TIME PCR IN BRAIN TUMOR

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CERTIFICATE

This is to certify that the dissertation entitled **"Investigation Of Yb-1 Gene Expression By Syber-Green Based On Real-Time Pcr In The Brain Tumor."** is the bonafide record of research work done by Mr. Muhammad Redzwan bin Sidik (87404) during the period from July 2008 to October 2008 under my supervision.

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Abstract

Cancer generally is a genetic disease that can cause alteration in specific gene in which a group of cells display uncontrolled growth, invasion and metastasis. YB-1 Gene Y-box binding protein (YB-1) is a member of the cold-shock domain (CSD) protein superfamily and involved in many cellular functions, including transcriptional regulation, translational regulation, DNA repair, drug resistance and stress responses to extracellular signals. YB-1 gene can be found in a cytoplasm and nuclear cell. The YB-1 gene comprises 8 exons spanning 19 kb of genomic DNA and is located on chromosome 1p34. The aim of the study is to differential relatively quantitative the expression of YB-1 gene in brain tumor sample (meningioma) and normal brain sample (Astrocyte SVG 12). SYBR-Green 1 based Real-Time PCR was performed and data analysis using formula $2^{-\Delta\Delta Ct}$ has demonstrated that YB-1 gene was over-expressed by 2.86 fold in comparison to its expression in normal sample. This preliminary data is very important in the future experiment for expression of YB-1 gene showed the potential as a biology marker for detection of brain tumor.

Abstrak

Kanser adalah penyakit genetik yang disebabkan berlaku pengubahan gen specifik dalam sel dimana kumpulan sel ini menunjukkan pembahagian sel tidak terkawal, menceroboh serta metatasis. Terdapat banyak gen yang boleh digunakan sebagai penanda biologi kanser seperti Gen YB-1. "Y-Box Binding Protein" (YB-1) adalah ahli keluarga protein "cold-shock domian"(CSD) dan terlibat dalam banyak fungsi sel melibatkan pengawalan transkriprasi, pengawalan translilasi, pembaikian DNA,ketahanan terhadap ubat, tindak balas tekanan kepada tanda diluar sel. Gen YB-1 boleh di jumpai di sitoplasma and nuklear sel. Ia mengandungi 8 exon dan mempunyai jarak genomik DNA 19 kb dan terletak pada chromosom 1p34. Matlamat kajian ini untuk membezakan secara relatif pengekspresian gen YB-1 di dalam sampel kanser otak(Meningioma) dan sampel otak yang normal(Astrocyte SVG 12). SYBR-Green 1 berdasarkan Real-Time PCR telah dilakukan dan analysis data mengunakan formula $2^{-\Delta\Delta Ct}$ menunjukkan gene YB-1 telah melebihi pengekspresian sebayak 2.86 kali berbanding pengekspresian sampel normal

Data awal ini sangat berguna dalam eksperimen akan datang untuk pengekspresian gen YB-1 sebagai penanda biologi untuk pengesahan kanser otak.

CHAPTER I

INTRODUCTION

1.1 Introduction to Cancer

Cancer also know as malignant or neoplasm in the medical term is a class of diseases in which a group of cells display uncontrolled growth, invasion, and sometimes metastasis. These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, do not invade or metastasize. Most cancers form a tumor but some like leukemia it does not happen.

Cancer is a genetic disease because it can be traced to be an alteration within specific genes but it is not an inherited disease. Due to genetic alteration, cancer cell will uncontrolled proliferation, producing malignant tumor that invade surrounding healthy tissue.

There are two general classes of genes that are related to genetic abnormalities of cancer. Oncogenes is a typically activated in cancer cells, giving those cells new properties, such as hyperactive growth and division, protection against programmed cell death, loss of respect for normal tissue boundaries, and the ability to become established in diverse tissue environments. Tumor suppressor genes are then inactivated in cancer cells, resulting in the loss of normal functions in those cells, such as accurate DNA replication, control over the cell cycle, orientation and adhesion within tissues, and interaction with protective cells of the immune system (Kumar , 1999).

Alterations of several tumor suppressor and oncogenes have been identified as being critical to the initial steps of transformation and progression to cancer (Christine . *et al* 2005). Associated alterations in cell cycle regulation and various growth factor signaling pathways are being dissected for their contributions to cancer progression.

1.1.2 Brain Tumor

Brain tumors are the leading cause of cancer related mortality in children. Pediatric grade IV astrocytomas (pediatric glioblastoma [pGBM]), non-neuronal tumors originating from the astrocytic lineage, around 15% of all pediatric brain tumors and have a 3-year survival of less than 20% and high morbidity (Faury *et al*,2007)

Adult GBM (aGBM) is the most common brain tumor that cause to death. Secondary GBM occur in adults aged younger than 40 years, evolve from low-grade astrocytomas. Primary GBM targets older patients and exhibits gain of function mutations of *EGFR*. Both forms are indistinguishable to pathologists, and share aberrations of the p53 and retinoblastoma (RB) pathways and similar prognosis (Maher *et al*, 2001, Louis *et al*, 2001)

Brain tumors have various shapes and sizes with different ways of behaving. It can grows within and originating from the brain parenchyma.. However, tumors arise from two sources which is a structures adjacent to the brain which can compress and distort it which include tumor meningiomas (Figure 1.1.2a) arising from the meninges, schwannomas from the cranial nerves, and adenomas from the pituitary gland. Another source is a metastatic tumors (Figure 1.1.2b) that originate from outside the CNS. The

commonest sources of origin are carcinomas of the breast, lung and kidney, and malignant melanoma. Some other common malignancies (e.g. prostate, bowel) only rarely metastasize to the brain. (Henry Marsh *et al*, 2007) .The classification of brain tumours is shown in table 1.1.2.

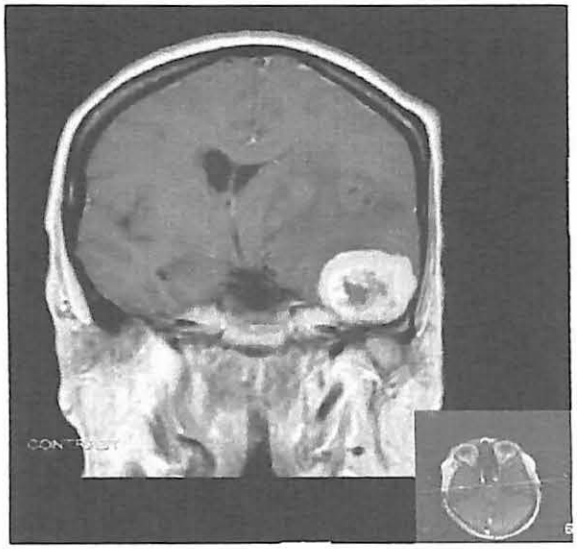

Figure 1.1.2a: Benign meningioma

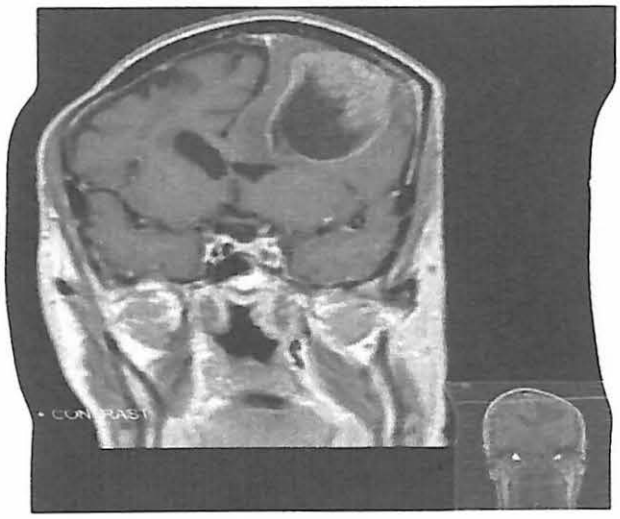

Figure 1.1.2b The commonest brain tumor an intrinsic malignant tumor that could be a glioma or metastasis

(Source: Henry ,2007)

Table 1.1.2: Classification of Brain Tumor

Classification of Brain Tumor
Tumor aring primarily from the brain • **Gliomas** **-Astrocytomas Oligodendrogliomas Ependymomas** • **Primary cerebral lymphoma** • **Choroid cerebral lymphoma** • **Choroids plexus papalilomas** • **Haemangioblastomas**
Metastatic tumors
Meningiomas
Pituitary Adenomas
Tumors of the skull

1.2 Statistic and prevalence of cancer in Malaysia (2003)

1.2.1 Overall Cancer Incidence

A total of 21,464 cancer cases were diagnosed among Malaysians in Peninsular Malaysia in the year 2003, comprising 9,400 males and 12,064 females. The National Cancer Registry received 42,985 cancer notifications of Malaysian residents in 2003 of which 23,746 were unique incident cancer cases. Of the 23746 cases, 22622 cases had histological verification thus 95.3 % of the cases had histological verification. The 2003 cancer incidence results presented in the rest of this report refer only to Peninsular Malaysia.

The crude rate for males was 97.4 per 100,000 population and 127.6 per 100,000 population for females. The age standardized incidence rate for all cancers in the year 2003 was 134.3 per 100,000 males and 154.2 per 100,000 females.

Cancer occurred at all ages. The median age at diagnosis for cancer in Malaysian males was 59 years and 53 years for Malaysian females. The 5 most frequent cancers in children (0-14 years old) were leukaemia, cancers of the brain, lymphoma, cancers of the connective tissue and kidney. In the group of young adults (15-49 years old), the common cancers were nasopharynx, leukaemia, lymphoma, lung, colon and rectum in men, and cancers of the breast, cervix, ovary, uterus, thyroid gland and leukaemia in women. In older subjects (50 years old and above), cancers of the lung, colon, rectum, nasopharynx, prostate and stomach were predominant among men, while cancers of the breast, cervix, colon, uterus, lung and rectum occurred commonly in women.

1.2.2 Brain and other nervous system cancers incident in Peninsular Malaysia 2003

In Peninsular Malaysia on 2003, there are 468 cases of brain and other nervous system cancers that had been reported. Number of male cases is 249 which are higher than female with 219 cases. Different percentage of both sex who get brain and other nervous system cancers is 6.4 % where male is 53.2% and female is 46.8%. Whereas incident per 100,000 populations (CR) of male is 2.6 and female is 2.3.

Table 1.2.2:Brain and other nervous system cancers incident per 100,000 population (CR) by peninsular Malaysia 2003

Sex	No	%	CR
Male	249	53.2	2.6
Female	219	46.8	2.3
Total	468	100	2.4

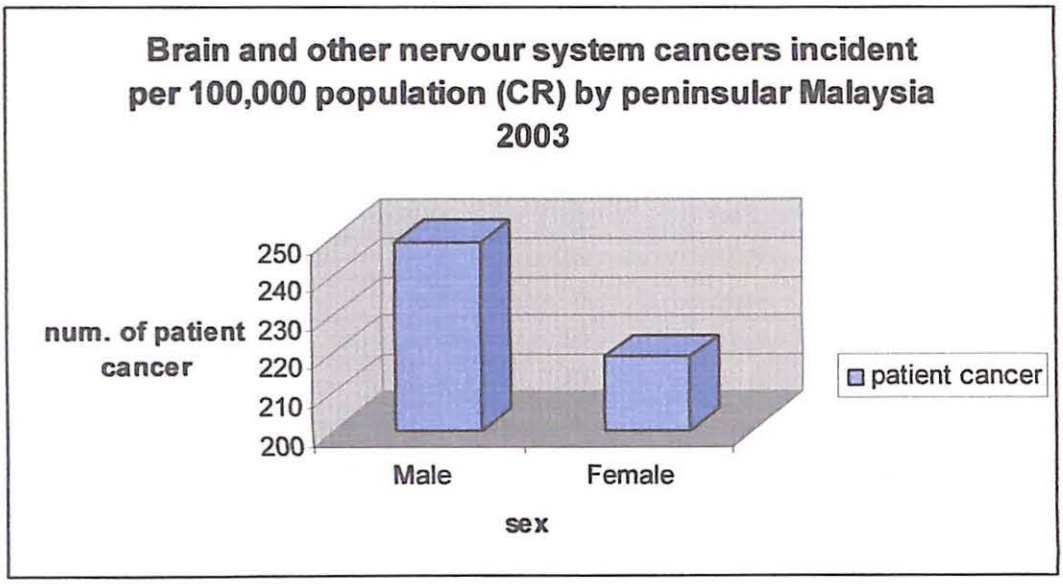

Figure 1.2.2: Brain and other nervour system cancers incident per 100,000 population (CR) by peninsular Malaysia 2003

1.2.3 Brain and Other nervous System. Age Specific Cancers Incident by sex, Peninsular Malaysia 2003

In 2003, there are 468 cases of brain and other nervous system had been reported. Based on the specific age between male and female, group age 30-39 show the higher number cases for male whereas for female, the higher cases reported at the specific group 30-39 and 50-59.

Table 1.2.3a shows that numbers of male cases relatively higher compare to the female. The higher number cancer patient for male is 43 cases whereas for female is 36 cases. However, at the age of 70 year old above show that both sex have low number of cancer cases.

Table 1.2.3a: Brain and Other Nervous System. Age Specific Cancers Incident per 100,000 populations (CR) by sex, Peninsular Malaysia 2003

	Male			Female			
Age(year)	No	%	CR	No	%	CR	
0-9	33	13.3	1.5	32	14.6	1.5	
10-19	38	15.3	1.9	31	14.2	1.6	
20-29	34	13.7	2.2	26	11.9	1.7	
30-39	43	17.3	3.2	36	16.4	2.7	
40-49	33	13.3	2.9	26	11.9	2.3	
50-59	26	10.4	3.5	36	16.4	5	
60-69	29	11.6	7.3	22	10	5.3	
70+	13	5.2	6	10	4.6	3.7	

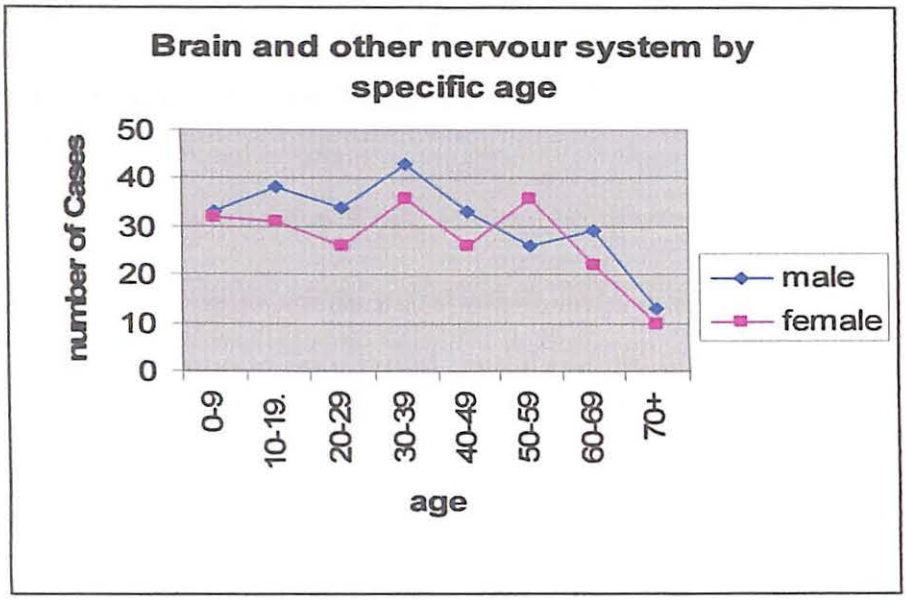

Figure 1.2.3b: Brain and Other nervous System. Age Specific Cancers Incident per 100,000 populations (CR) by sex, Peninsular Malaysia 2003

1.3 The Classification of Brain Tumor

1.3.1 The Classification of Brain Tumor based on World Health Organization (WHO)

Based on the 4th edition of the World Health Organization (WHO) classification of tumors of the central nervous system, published in 2007, it can be divided into 7 group which is Tumors of Neuroepithelial Tissue, Germ Cell Tumors, Tumors of Cranial and Paraspinal Nerves,Tumors of the Sellar Region, Tumors of the Meninges, Metastatic Tumors, Lymphomas and Haemopoietic Neoplasms .These classifications is based on the consensus of an international Working Group of 25 pathologists and geneticists, as well as contributions from more than 70 international experts overall, and is presented as the standard for the definition of brain tumors to the clinical oncology and cancer research communities world-wide.

An entity had to be characterized by distinctive morphology, location, age distribution and biologic behaviour, and not simply by an unusual histopathological pattern.(Louis *et al,* 2007) 1. Variants were defined as being reliably identified histologically and having some relevance for clinical outcome, but as still being part of a previously defined, overarching entity. Finally, patterns of differentiation were considered identifiable histological appearances, but that did not have a distinct clinical or pathological significance. The classification is shown the table 1.3 1which include type of cancer and it grade.

Table 1.3.1 : World Health Organization (WHO) classification of tumours of the central nervous system, published in 2007

Classification Of Tumours Of The Central Nervous System By WHO	
Tumours Of Neuroepithelial Tissue	Astrocytic tumours Oligodendroglial tumours Oligoastrocytic tumours Ependymal tumours Choroid plexus tumours Other neuroepithelial tumours Neuronal and mixed neuronal-glial tumours Tumours of the pineal region Embryonal tumours
Tumours Of Cranial And Paraspinal Nerves	Schwannoma (neurilemoma, neurinoma) Cellular Plexiform Melanotic Neurofibroma Plexiform Perineurioma Perineurioma, Malignant perineurioma Malignant peripheral nerve sheath tumour (MPNST)
Tumours Of The Meninges	Tumours of meningothelial cells Mesenchymal tumours Primary melanocytic lesions Other neoplasms related to the meninges
Lymphomas And Haematopoietic Neoplasms	Malignant lymphomas Plasmacytoma Granulocytic sarcoma
Germ Cell Tumours	Germinoma Embryonal carcinoma Yolk sac tumour Choriocarcinoma Teratoma Mature Immature
Tumours Of The Sellar Region	Craniopharyngioma Adamantinomatous Papillary Granular cell tumour Pituicytoma Spindle cell oncocytoma of the adenohypophysis
Metastatic Tumours	

1.3.2 WHO grading

The WHO grading of CNS tumors establishes a malignancy scale based on histologic features of the tumor .Histological grading is a means of predicting the biological behaviour of a neoplasm. In the clinical setting, tumour grade is a key factor influencing the choice of therapies, particularly determining the use of adjuvant radiation and speciWc chemotherapy protocols. The WHO classification of tumours of the nervous system includes a grading scheme that is a 'malignancy scale' ranging across a wide variety of neoplasms rather than a strict histological grading system. Table 1.3.2 shown the summary of the tumours of the central nervous system grade based of World Health Organization (WHO).

Table 1.3.2: Summary of the tumors of the central nervous system grade based of World Health Organization (WHO)

WHO Grade	Summary
WHO GRADE I	includes lesions with low proliferative potential, a frequently discrete nature, and the possibility of cure following surgical resection alone
WHO GRADE II	includes lesions that are generally infiltrating and low in mitotic activity but recur. Some tumor types tend to progress to higher grades of malignancy
WHO GRADE III	includes lesions with histologic evidence of malignancy, generally in the form of mitotic activity, clearly expressed infiltrative capabilities, and anaplasia.
WHO GRADE IV	includes lesions that are mitotically active, necrosis-prone, and generally associated with a rapid preoperative and postoperative evolution of disease