CHAPTER 1

INTRODUCTION

Cancers often begin from a single cell (Salk et al., 2010). The accumulation of inherited and somatic mutations (Knudson, 1971; Ponder, 1992) in proto-oncogenes or tumor suppressor genes leads to cancer (Vogelstein & Kinzler, 2002). In contrast to activating mutations that induce oncogenic alleles from proto-oncogenes, tumor suppressor genes are inactivated by loss-of-function mutations in malignant cells (Vogelstein & Kinzler, 2002; Weinberg, 1991). In line with this, Knudson (1971) proposed his "two-hit hypothesis" that elucidated at molecular level how retinoblastoma develops. According to the hypothesis, two somatic mutations are required within the same cell in sporadic cases whereas only one further (somatic) mutation is required in a predisposed cell with an inherited mutation for tumor formation. As the cancer risk not only depends on environmental exposure but also on genetic makeup, in certain individuals the inherited factor plays a significant role owing to the underlying critical mutation being present in every cell (Ponder, 1992). Indeed, these people have a head start in the course of time to accumulate mutations (Knudson, 1971; Salk et al., 2010).

The most common tumor suppressor genes with mutations within neoplasms are *RB1* and *TP53* (Kemp et al., 2008). *RB* or *RB1* gene is also known as the retinoblastoma susceptibility gene (Chan, 2001). Discovered in 1986, *RB1* was the first human tumor suppressor gene to be identified in families with retinoblastoma (Donovan et al., 2006). In fact, defects in both alleles of the *RB1* gene result in retinoblastoma (Knudson, 1971; Shin & Grossniklaus, 2011). Thus, children who inherit one defective copy of the *RB1* gene have an increased susceptibility to retinoblastoma (Donovan et al., 2006).

Retinoblastoma is the commonest primary ocular malignancy in children below five years of age (Canty, 2009; Shin & Grossniklaus, 2011). The tumor arises in the retina (Aerts et al., 2006; Rong, 2000). The incidence is 1 in 15,000 to 1 in 20,000 live births although the rate may vary in different countries (Jamalia et al., 2010; Shin & Grossniklaus, 2011; Rong, 2000). Retinoblastoma can occur either as a unilateral (one eye affected) or bilateral (both eyes affected) disease. Almost all bilateral tumors are considered hereditary (Parsam et al., 2009). However, only 10 - 15% of bilateral retinoblastoma cases have a family history, the rest being *de novo* germ-line mutations that may be transmitted to future generations (Quah, 2005).

To date, very few studies have been done to reflect the incidence of retinoblastoma in Malaysia despite the frequency of new patients that is on the rise every year as reported by Hospital Kuala Lumpur, the main referral centre for retinoblastoma in Malaysia (Jamalia et al., 2010). Based on the National Eye Database review, the exact local incidence rate, prevalence and clinical characteristics of retinoblastoma remain unknown (Ishak et al., 2010; Jamalia et al., 2010; National Eye Database, 2012). The National Cancer Registry report for the year 2006 by Ministry of Health Malaysia had signified high incidence rate of eye cancer among males (9 in 100,000 of population) than females (2 in 100,000 of population) in Peninsular Malaysia. On the other hand, a study conducted by Menon (2009) reviewed the features of clinical presentation, treatment and consequences for local children with retinoblastoma. The review report was in concordance with Jamalia et al. (2010) in which retinoblastoma in Malaysia is still marked by predominantly extraocular (exterior to the eye) malignancy due to delayed presentation and high level of ignorance.

Retinoblastoma is often fatal if left untreated (Shin & Grossniklaus, 2011) or if diagnosis is delayed (Jamalia et al., 2010). However, 95% of patients can survive if they are treated before the tumor spreads beyond the eye (Field et al., 2007; Shin & Grossniklaus, 2011). As a related concern, it is important to identify *RB1* mutations that lead to the disease to improve the clinical management of Rb patients and to ensure proper genetic counselling to the family (Aerts et al., 2006; Lohmann, 1999). For this, molecular methods offer high sensitivity in identifying *RB1* mutations which in turn provide key information to affected families and clinicians (Lee et al., 2009). In order to decrease the number of patients with advanced extraocular disease and to offer less aggressive treatment with better outcomes, early detection of carriers by employing molecular methods such as PCR and sequencing are preferred (Salk et al., 2010). An early diagnosis of Rb not only contributes to improved vision outcomes but also mitigates the overall cost of healthcare (Lee et al., 2009).

The development of secondary malignancy in many retinoblastoma survivors owing to *RB1* germ-line mutation irrespective of treatment explains the need for predictive molecular diagnostics. Upon confirmation of constitutional origin of mutations, patients are included in high risk group for whom careful follow up is recommended for they are more likely to develop a second tumor in adult life compared to non-carriers (Braggio et al., 2004). Fernandez et al. (2007) and Lohmann (1999) had highlighted that children without *RB1* mutations need not undergo ophthalmic examination under anaesthesia during the first two years of life as opposed to *RB1* mutation carriers. In other words, this improves quality of life by reducing the intensity of clinical surveillance for young relatives of proband who are proven to not carrying the proband's *RB1* mutation (Lee et al., 2009). Richter and colleagues (2003) recapitulated the significance of molecular screening that helps to decrease or bypass the need for clinical examination.

As outlined by Aerts et al. (2006), molecular tests for genetic analysis may encompass: 1) direct examination for a constitutional mutation executed on the constitutional DNA, 2) indirect proof of the allele carrying the mutation in cases of familial history, and 3) tumor loss of heterozygosity evaluation. This study adopted the first method by which mutation detection rate is likely to be high in hereditary cases (Houdayer et al., 2004). Nevertheless, the large size of the *RB1* gene (approximately 200 kb) with its multiple exons and the heterogeneity of mutations usually complicate molecular screening for presymptomatic diagnosis and for carrier detections (Fernandez et al., 2007; Retinoblastoma: Molecular Genetics, n.d.). The heterogeneity of *RB1* mutations are due to gross rearrangements (20%) or small mutations (80%) in most of the 27 exons of the *RB1* gene (Fernandez et al., 2007; Horsthemke, 1992). In view of this, exon-by-exon examination for mutations is favoured for accurate and comprehensive molecular findings (Fernandez et al., 2007). With reference to the above-mentioned, this study incorporated molecular techniques such as simple PCR and direct DNA sequencing followed by exon-by-exon screening and analysis of mutations present in the *RB1* gene of Malaysian patients with retinoblastoma.

1.1 Significance of the Study

Limited studies have been done to reveal insights into molecular genetics of *RB1* gene in Malaysian patients with retinoblastoma. Additional studies and further approaches are thus required to investigate the mutations that give rise to Rb in Malaysian children. In considering the fact that none of the previous studies had targeted the identification of *RB1* mutations in Malaysian patients with retinoblastoma, this research was initiated with the aim of identifying *de novo* mutations in the *RB1* gene of patients with retinoblastoma who sought treatment at UMMC. The research was basically designed to study sporadic Rb case series of UMMC, received from April 2011 to June 2012. The study focused on the type of mutations that might have attributed to the phenotype or disease.

Identification of heritable germ-line mutations by PCR and direct sequencing may help render accurate genetic counselling to at-risk relatives (Brown, 2006; Chen et al., 2003). Being sensitive and reliable, simple PCR and sequencing which were employed in this study facilitated early detection of at-risk relatives and obviate the need for routine clinical examination. This also helps to decrease the financial burden of the affected family in coping with the management of the disease (Parsam et al., 2009). The results obtained from this study may be helpful to indicate possible future implications to the patient and to the respective family members. Since the mutation identification rate is considerably high in hereditary patterns and most mutations are known to be scattered along *RB1*, frequent mutation site or common mutations that are identified could be advantageous in devising predictive genetic testing (Brown, 2006; Valverde et al., 2005). Furthermore, early diagnosis would be able to mitigate the morbidity and mortality rate caused by eye cancer apart from facilitating the physicians to execute prompt treatment for better visual outcome as well as to preserve vision in affected patients (Macdonald & Ford, 1991). The identification of genetic aberrations in *RB1*

also aids in alerting the patients and their families to take precautions against occurrence of second malignancies. Besides offering a cost-effective option (Parsam et al., 2009), early detection of *RB1* mutations may also help improve prognosis and quality of life of Malaysian patients with retinoblastoma.

1.2 Research Questions

The present study is designed to answer the following research questions:

1- Does a patient with unilateral or bilateral retinoblastoma with no family history of the disease possess germ-line mutation in the *RB1* gene?

2- Is the presence of germ-line mutation in the *RB1* gene associated with bilateral phenotype of the disease?

1.3 Objectives of the Study

1- To identify *de novo RB1* germ-line mutations in patients with retinoblastoma from University Malaya Medical Centre (UMMC)

2- To correlate the presence of germ-line mutation in the *RB1* gene with the incidence of bilateral retinoblastoma