CHAPTER I

INTRODUCTION

1.1 GENERAL INTRODUCTION ON CANCER

Cancer is a diverse group of diseases that share abnormal cell proliferation as a leitmotif. Essentially, failure of normal cells to regulate their growth causes them to be transformed and become cancerous. In 2000, Hanahan and Weinberg proposed six 'cancer hallmarks' – defined as the biological capabilities that are acquired by the transformed cells. The original cancer hallmarks were initially defined as sustaining cell proliferation, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis, and resisting cell death (Hanahan & Weinberg, 2000). In addition, two emerging hallmarks have been recently proposed: reprogramming of cellular metabolism, and resistance to antagonisms from the immune systems. Underneath these hallmarks are two factors that precipitate their acquisition by the cells: the instability of the genome, as well as the inflammatory state of the neoplastic lesions (Hanahan & Weinberg, 2011).

Microarray has been widely used to identify genes that are differentially expressed between normal and cancerous cells. However, a lot of dysregulated genes are still of uncertain significance to the transformation process. It is necessary to examine the impact of altering the level of a gene on the cancer hallmark traits in order to implicate it as a true 'driver' of oncogenesis, instead of a mere 'passenger' event. Ultimately, for a gene that is overexpressed in cancer, the impairment of the hallmark capabilities as a result of the inhibition of the gene function could be the basis of therapeutic development. In addition, the identification of a gene that does not directly correlate with the hallmark traits but correlate instead with a disease parameter would qualify the gene to be developed as a biomarker. The results of these studies would advance the still rudimentary understanding of the molecular underpinnings of cancer, in the hope that this knowledge would translate to better disease management in the clinic.

1.2 NASOPHARYNGEAL CARCINOMA

1.2.1 Background

Nasopharyngeal carcinoma (NPC) is a type of cancer that arises from the epithelial lining of the nasopharynx (hence nasopharyngeal carcinoma), the uppermost area of the throat. It usually arises from the lateral nasopharyngeal recess of the Rosenmüller fossa. NPC is distinct from other types of head and neck cancer on the grounds of incidence, risk factors, clinical behavior, as well as association with the Epstein - Barr virus (EBV).

1.2.2 Histopathology

Histopathological classification of NPC, as proposed by the World Health Organization (WHO) in 1978, categorizes the disease into three types based on the degree of the tumor cell differentiation. Type I consists of well differentiated keratinizing squamous carcinoma cells, with occurrence of intercellular bridges and general cell keratinization. Types II and III consists of non-keratinizing cells, with Type II formed by moderately-differentiated cells, while Type III being made up of undifferentiated cells.

1.2.3 Epidemiology

NPC is extremely rare in most countries, with incidence less than 1 case in 100,000 people. Cases that do occur in Western Europe or in the United States are mainly of the differentiated Type I diseases (Brugere et al., 1978; Chow et al., 1993; Vaughan et al., 1996). However, outstandingly high incidence among people of Southern Chinese descendants are observed both in their homeland and the place where they migrate to; incidence in Southern Chinese cities Guangzhou and Zhongshan are 22 and 27 respectively, while in Hong Kong is

18, and Singapore is 11 per 100,000 population (Curado et al., 2007). Moderately high incidence of around 4 cases in 100,000 population meanwhile occurs among the North Africans, the Inuits in Greenland, as well as among the Filipinos. In all affected populations the ratio of male to female is consistently about 3 to 1.

In our local context, NPC is the fifth most common cancer overall and the third most common among Peninsular Malaysia males (Figure 1A). In addition, among Peninsular men of the productive age bracket from 15 to 49 years, NPC is the most frequent cancer (Figure 1B) (Omar et al., 2006). Moreover, its incidence among the indigenous Bidayuh people of Sarawak is the highest known in the world (Devi et al., 2004).

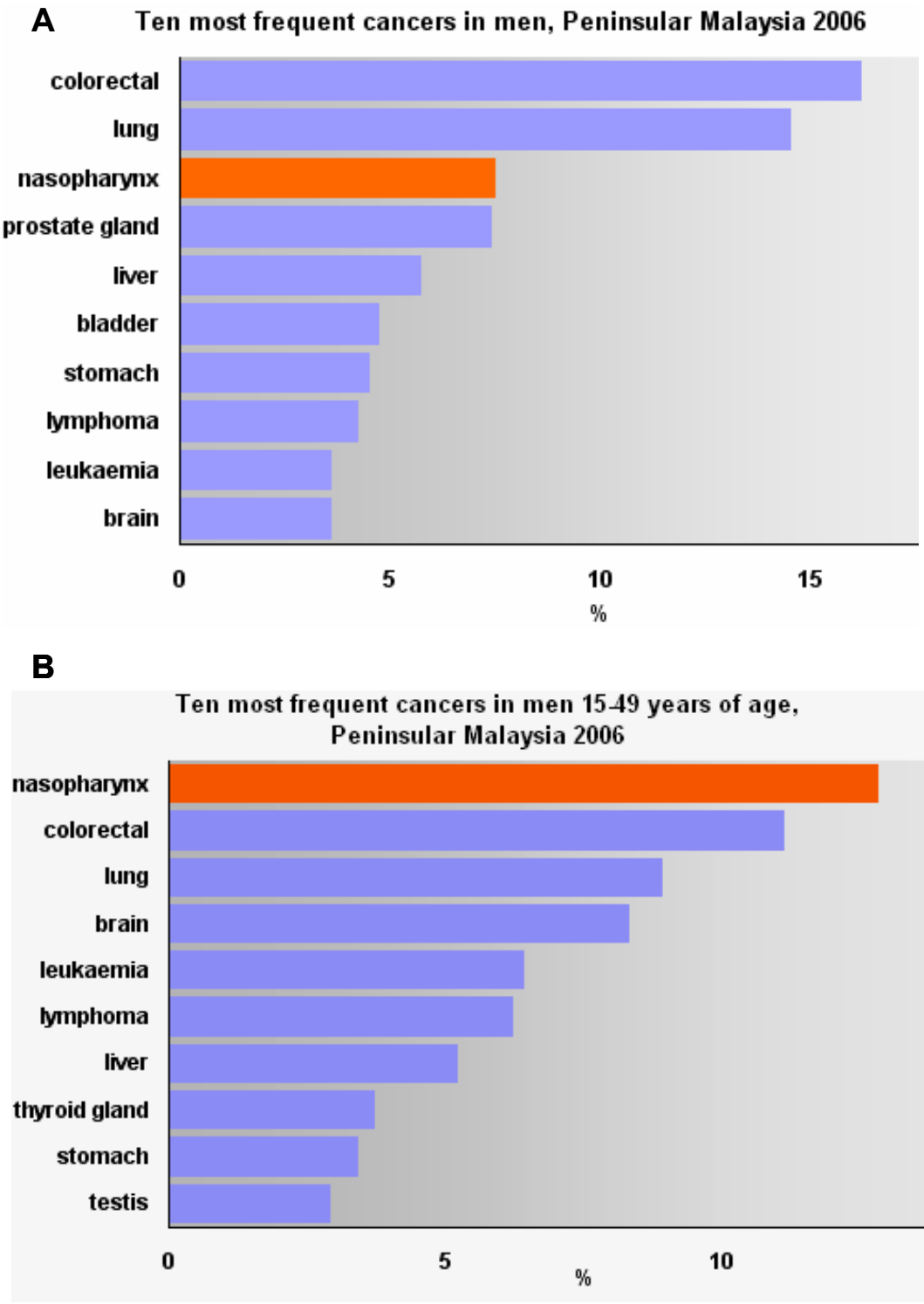

Figure 1.1 | NPC incidence in Peninsular Malaysia, 2006. (A) NPC is the third most frequent cancer among Peninsular Malaysia males, among which in 15-49 years old age bracket the cancer is the most frequent. Image adapted from the National Cancer Registry 2006 (Omar et al., 2006).

1.2.4 Etiological Factors

1.2.4.1 Genetic

The most well-studied genetic susceptibility factor of NPC is the HLA locus. HLA genes encode for cell-surface proteins that present peptides from the inside of a cell, including those of foreign (e.g viral or bacterial) origin. The presentation of these peptides is necessary for correct immune function. HLA involvement in NPC was initially thought to be as a result of its inability to present EBV epitopes correctly, but there is no evident difference between the EBV peptide binding by the different HLA that are associated with disease risk (Li et al., 2009). The association of HLA with an increased risk in NPC has been documented as early as in the 1970s among the Chinese (Simons et al., 1974). Among the population HLA alleles A2, B14 and B46 confer increased risk while A11, B13 and B22 confer negative risk for the disease (Goldsmith et al., 2002). However, even though some alleles carry an increased risk for NPC in one population, the same alleles could carry a decreased risk for the disease in another (Boussen et al., 2005).

In addition, other studies have also pointed to the presence of different genetic elements other than HLA genes, close to the MHC region in chromosome 6 as a genetic susceptibility factor (Ooi et al., 1997). Genome wide association studies meanwhile have further implicated novel susceptibility loci that code for ITGA9, GABBR1, TNRSF19, MDS1-EVI1 and CDKN2A-CDKN2B (Ng et al., 2009; Tse et al., 2009; Bei et al., 2010).

1.2.4.2 Diet

The importance of diet as an etiological factor was first recognized with the finding that consumption of salted fish that contains nitrosamines carries a strong predisposition for the disease development among the Southern Chinese (Fong & Walsh, 1971; Zou et al.,

1992). Specifically, the consumption of the salted fish during early childhood and at high regularity correlates with an increased risk of developing NPC (Armstrong et al., 1983; Yu et al., 1983; Yu & Henderson 1987; Guo et al., 2009). Nitrosamine-containing food remains a predisposing risk even in non-endemic population such as in the United States, where the study subjects are Caucasian and African American (Farrow et al., 1998). In addition, the expression levels of nitrosamine metabolism pathway components are also down-regulated in the cancerous tissue as reported in a global gene expression study (Dodd et al., 2006). Further support has come from the experimental evidence in animals that showed the rodents develop carcinomas of nasal cavities when fed with salted fish (Huang et al., 1978; Yu et al., 1989). A derivative of nitrosamines, *N,N'*-dinitrosopiperzine could reproducibly induce NPC in rats with no cancer in other organs was observed (Pan et al., 1978). Apart from nitrosamine, consumption of food that contains butyric acid, such as rancid butter, sheep fat and *quaddid* (preserved meat) was also associated with an increased NPC incidence among the North Africans (Feng et al., 2007) by activating the lytic cycle of EBV (Bauer, 1983).

1.2.4.3 Epstein - Barr Virus

Epstein - Barr virus is a human γ-herpesvirus with a double-stranded linear DNA genome of about 170kbp. It latently infects 90% of world human population. EBV presence is detectable as early as in high grade pre-invasive (severe dysplasia and carcinoma *in situ*) nasopharyngeal tissue. The clonal nature of the viral genome in the NPC cells also supports the postulate that the viral infection occurs very early during the disease progression (Pathmanathan et al., 1995).

Virtually all undifferentiated NPC cases (Types II and III) are associated with EBV. The latent form of the virus always exists in the cancerous cells but were absent in the

surrounding normal tissues. EBV infection in NPC cells falls under latency Type II, where all cells express viral genes EBNA1, EBER1, EBER2, LMP2A and LMP2B, while LMP1 is present in most NPC tumor cells.

1.2.5 Clinical Presentation

Over 80% of the patients come at late stage because of the non-specific nature of the nasal and aural symptoms, as well as the difficulty for clinical assessment of the nasopharynx (Pua et al., 2008). The presenting clinical symptoms can be divided according to the anatomical sites that are invaded and affected as the tumor develops: 1) nasopharyngeal tumor mass leading to epistaxis (nosebleed), nasal obstruction and nasal discharge; 2) Eustachian tube malfunction leading to tinnitus (ringing in the ear) and deafness; 3) skull-base erosion and cranial nerve palsy leading to headache, diplopia (double vision) and numbness; and 4) metastases to lymph nodes leading to lymphadenopathy (enlarged lymph nodes), characterized by gross neck mass (Wei & Sham, 2005). Patients come to the clinic with complaints of cervical lymphadenopathy as the most common symptom, followed by nasal, aural and other ophthalmo-neurologic symptoms (Chan et al., 2002; Pua et al., 2008).

1.2.6 Treatment

Stage I and IIa diseases are most often treated with radiotherapy. Whenever possible, 3D conformal therapy or even Intensity-Modulated Radiation Therapy (IMRT) is preferred over standard 2D conventional radiotherapy (Chao et al., 2000). As earlier stage tumors are highly radiosensitive, overall survival rate after radiotherapy as high as 85% for Stages I and II could be achieved. However, the overall survival rate falls to 55% for Stages III and IV due to local failure and distant metastasis (Teo et al., 1996). Locoregionally advanced (Stages IIb

to IV) diseases are treated with a combination of chemotherapy and radiotherapy (Al-Sarraf et al., 1998; Lin et al., 2003). Adjuvant chemotherapy usually involves cisplatin and 5-flurouracil while concurrent chemotherapy involves cisplatin alone. Currently, concurrent chemoradiotherapy seems to be more efficacious (Wei & Sham, 2005). Although long-term diseases-free survivors for patients with metastases have been reported, treatment of metastatic nasopharyngeal carcinoma is essentially palliative (Fandi et al., 2000).

1.3 CURRENT MOLECULAR UNDERSTANDING OF NPC

1.3.1 Cytogenetic Studies

During NPC development the nasopharyngeal epithelial cells suffers from progressive and incremental genomic change, suggesting that the genetic change is a progressive multi-step event (Chen et al., 1999), rather than a single catastrophic chromotripsis event (Stephens et al., 2011). 3p and 9p21 chromosomal deletions are probably the earliest recognizable events in NPC. These lesions occur in normal adult epithelium in high risk population, leading to specific inactivation of RASSF1A (at 3p) and p16 (at 9p) tumor suppressors (Chan et al., 2000; Chan et al., 2002; Shao et al., 2002), even though these tumor suppressors can also be inactivated via methylation (Lo et al., 2001). These events might incline nasopharyngeal cells to latent EBV infection (Chan et al., 2004). Gain in chromosome 12 could be another important early event, and may represent a different subclass of NPC from those that carry 3p loss (Shih-Hsin Wu, 2006).

Some patterns of genomic lesion are consistent in both early and late cases, with a trend for these lesions to occur at a higher frequency in advanced cases (Li et al., 2006). Gain of 3p, 8q, 12p, 12q, and loss of 14p and 14q commonly seen across the stages suggests that these lesions might be essential lesions for the disease progression, or that these are

fundamental lesions that actually occur during early oncogenesis (Li et al., 2006). Advanced NPC cases exhibit a strong association with gain of 1q, 8q, 18q and loss of 9q, 11q, 16q (Chien et al., 2001; Fang et al., 2001; Shao et al., 2002; Li et al., 2006). Gain at 1q is seen at much higher frequency in Mediterranean cases than in Asian cases (Rodriguez et al., 2005). Even though some lesions are frequently observed in cases regardless of stages, none of them are seen in more than 30% of all cases (Li et al., 2006).

For recurrent tumors, 11q13 amplification which can already be observed in primary tumors becomes more frequent with no new identifiable lesions, suggesting that the tumor could utilize other mechanisms to generate the genetic diversity required for survival (Chen et al., 1999). For neck lymph node metastases, more extensive alterations in the form gains of 8p and 8q as well as loss of 9p, 16p, 7q, 20q, 21p, 21q, 22q are detected, implying that the primary tumor cells had to undergo more genomic changes for the metastases to become successful (Yan et al., 2005).

From the information on genomic alterations of NPC tumors, it has been postulated that the disease pathogenesis possibly does not follow a simplistic and rigid linear model. A different approach involving distance-based and branching-tree methods to construct more general tree-like models have been proposed as an alternative: NPC can be classified into two groups, one marked by gains at 12p12 and 8q22, and the other group (3p-deficient NPC) by gain in 1q22-32 plus loss in 3p26, 11q22, 14q24 (Huang et al., 2004). Another study further proposed a refined version of this model, by suggesting that there are at least two subclasses of 3p-deficient derived NPC, one marked by gain in 1q plus loss of 9p and 13q, while the other marked by loss of 14q, 16q, 9q, and 1p (Shih-Hsin Wu, 2006).

1.3.2 Molecular Alterations

1.3.2.1 Dysregulation of the Signaling Pathways

1.3.2.1.1 The Wnt Pathway

The Wnt signaling pathway is involved in a number of normal physiological processes including organ morphogenesis, maintenance of adult stem cell (Sugimura & Li, 2010), determination of cell polarity (Wallingford & Mitchell, 2011), and nervous system wiring (Salinas & Zou, 2008). Aberrant expression of Wnt pathway components has been repeatedly documented in NPC cases of different origins from global gene expression studies, suggesting that Wnt pathway dysregulation is critical for NPC progression regardless of ethnic origin (Shi et al., 2006; Sriuranpong et al., 2004; Zeng et al., 2007).

At the upstream level of the pathway and at the extracellular of the cell, the increased WNT5A ligand (Zeng et al., 2007) and the downregulation of an antagonist of Wnt proteins WIF-1 (Chan et al., 2007; Fendri et al., 2010) have been shown in NPC. Furthermore, Fzd7, a receptor of the Wnt proteins, has also been shown to be upregulated (Zeng et al., 2007). Beta-catenin is a downstream molecule of Wnt pathway that is translocated from the membrane to the nucleus and act as transcriptional coactivator on pathway activation. The protein was observed to be localized at the membrane of non-malignant nasopharyngeal tissues (Galera-Ruiz et al., 2011), but loses its membrane localization with corresponding increase in nuclear localization in NPC (Wang et al., 2009). The increase of beta-catenin in the tumor cells also correlates with higher tumor staging (Galera-Ruiz et al., 2011; Wang et al., 2009). In addition, a regulator of beta-catenin, GSK-3beta has been shown to be phosphorylated in NPC, which would result in its ability to antagonize beta-catenin transcriptional activation function (Morrison et al., 2004). Furthermore, oncogenic mutation

in beta-catenin, which increases its stability in other cancers, is a rare event in NPC (Li et al., 2004).

1.3.2.1.2 The EGFR Pathway

Various components of the EGFR signaling transduction pathway have been studied in NPC. The EGFR gene is amplified (Hui et al., 2002) and overexpressed in NPC (Fang et al., 2007). The expression of EGFR (Wang et al., 2006; Yang et al., 2009; Cao et al., 2011) and its active form pEGFR (Yuan et al., 2008) has been shown to be associated with poor prognosis. The expression of EGFR also correlates with disease staging (Chua et al., 2004; Yuan et al., 2008) and it persists in lymph node metastases (Huang et al., 2009).

Ras, Raf, and Erk belong to the same signaling cascade that could be affected by the signal from activated EGFR. RASSF2, a negative regulator of Ras is downregulated and its low expression correlates with lymph node metastasis (Zhang et al., 2007). Meanwhile, Raf kinase inhibitor protein (RKIP), a negative regulator of Raf, is differentially phosphorylated in NPC, and its downregulation was significantly correlated with advanced clinical stage, lymph node metastasis and radioresistance (Chen et al., 2008; Chen et al., 2009; Ruan et al., 2010).

However, despite the information pointing to the hyperactivation of the EGFR signaling pathway in NPC, a clinical trial using Gefitinib, a small molecule inhibitor of wild type EGFR shows that the drug has very limited activity in recurrent NPC cases (Ma et al., 2008), despite the fact that EGFR protein in NPC bears no mutation (Naji et al., 2010). Treatment with Sorafenib, a Raf/Erk inhibitor had only modest anticancer activity in NPC, despite the tumor showing a decrease in activate ERK after the drug treatment (Elser et al., 2007). The results from these two clinical trials suggest that, different from cancers for which

Gefitinib and Sorafenib have been successful, the dysregulation of the EGFR pathway and its components may confer a fundamentally different effect to NPC pathogenesis that is yet to be understood.

1.3.2.2 Overexpression of the DNA Repair Pathway Components

As high as about 93% genes that are annotated within the DNA repair pathways are overexpressed in NPC as reported in a global gene expression study (Dodd et al., 2006). Overexpression of a number of genes, including HMGB1, DNA-PKCs, ERCC1 and Topoisomerase IIα have been shown to correlate with disease staging, poor survival and poor prognosis (Xiang et al., 2004; Ding et al., 2008; Lee et al., 2010). In contrast, downregulation of several components of the DNA repair pathway, including ATM (Bose et al., 2009), ADPRT and HOGG1 (Liu et al., 2008) have also been observed.

A peculiar feature of NPC is the overexpression of intact P53, in contrast to the general observation that P53 mutation or downregulation are ubiquitous in most human cancers. The overexpression (Hsu et al., 2002; Agaoglu et al., 2004; Chen et al., 2004; Hoe et al., 2009) and the wild type nature (Spruck et al., 1992; Sun et al., 1992; Sheu et al., 2004; Hoe et al., 2009) of the protein has been well documented in NPC cases of various origins. Its expression is also associated with a better response to chemotherapy and better survival (Hsu et al., 2002). The reason for its overexpression in NPC is still unclear. Even more intriguing is that p53 overexpression might occur before EBV infection of the nasopharyngeal dysplasia (Gulley et al., 1998).

1.3.2.3 Dysregulation of the Cell Cycle Components

The dysregulated expression of G1-S transition phase components of the cell cycle has been consistently documented in global gene expression studies of NPC (Xie et al., 2000; Sengupta et al., 2006; Sriuranpong et al., 2006; Zeng et al., 2007). Cyclin D1 is overexpressed (Xie et al., 2000; Zhang et al., 2009; Acikalin et al., 2011) and amplified in NPC (Hui et al., 2005). The expression of cyclin D1 correlates with early local recurrence and poorer prognosis (Lai et al., 2002; Zhang et al., 2009), and has been proposed as a predictor to local recurrence after radiotherapy for NPC (Hwang et al., 2002). Furthermore, polymorphism of the gene promoter was associated with inherent susceptibility to NPC (Deng et al., 2002).

p27 (encoded by the CDKN1B gene) and p16 (encoded by the CDKN2A gene) proteins control the cell cycle progression at G1 by inhibiting cyclin D1/CDK4 and cyclin E1/CDK2 complexes. These proteins are downregulated at the early stage of NPC development, possibly after the nasopharyngeal hyperplastic lesions (Fan et al., 2006). Furthermore, low expression of p27 correlates with cranial nerve encroaching (Li & Zhang, 2003) and loco-regional recurrence (Hwang et al., 2003), while loss of p16 was related to post-radiotherapy recurrence (Hwang et al., 2002; Makitie et al., 2003).

1.3.2.4 Dysregulation of Global miRNA Expression Profile

There are three reports on the dysregulation of human miRNA profile in NPC (Sengupta et al., 2008; Chen et al., 2009; Li et al., 2011). It is possible to segregate NPC cases according to clinical stages based on global profile of miRNA alone (Li et al., 2011), an exhibit not possible using expression profile of non-miRNA transcripts (Zeng et al., 2007).

Some of the dysregulated miRNA which biological roles are relatively well-characterized warrant further attention.

A number of miRNAs are downregulated in NPC and function as tumor suppressors by regulating extracellular matrix protein expression (miR-29c; (Sengupta et al., 2008), and targeting the oncogene EZH2 (miR-26a; (Alajez et al., 2010; Lu et al., 2011). On the other hand, major oncomiRs that are overexpressed in NPC include miR-17-92 cluster, miR-155 and miR-141 (Chen et al., 2009). Polycistronic miR-17-92, also known as oncomir-1, is a strongly oncogenic family of miRNAs that inhibits differentiation as well as enhances proliferation, angiogenesis and cell survival (Olive et al., 2010). Mir-155 is pleiotropically involved in differentiation, immunity, inflammation, and its expression is also increased in a variety of cancers (Faraoni et al., 2009). The overexpression of miR-141 meanwhile increases cisplatin resistance in esophageal cancer cells (Imanaka et al., 2011).

Reports investigating the EBV miRNA transcriptome in NPC have revealed a consistent pattern of viral miRNA expression in NPC (Chen et al., 2009; Cosmopoulos et al., 2009; Zhu et al., 2009; Gourzones et al., 2010). BART miRNAs that may negatively regulates EBV proteins LMP1 (Lo et al., 2007) and LMP2A (Lung et al., 2009) are expressed abundantly in NPC tumors and are released into the blood (Cosmopoulos et al., 2009; Zhu et al., 2009; Chen et al., 2010; Gourzones et al., 2010). Meanwhile, prosurvival (Li et al., 2006; Desbien et al., 2009) BHRF1 miRNAs are not expressed in NPC cells (Cosmopoulos et al., 2009; Zhu et al., 2009; Chen et al., 2010). Finally, C666.1 being the only EBV positive cell line, shows viral miRNA expression that is generally representative for NPC tumors (Cosmopoulos et al., 2009).

1.3.2.5 Proteomics Studies

Four independent proteomic studies have identified ceruloplasmin and chloride intracellular channel 1 (CLIC1) as potential serum biomarkers for NPC. These proteins are also overerexpressed in the primary tumors (Doustjalali et al., 2006; Chen et al., 2008; Chang et al., 2009; Chang et al., 2010). Serum protein profiling was also shown to be able to discriminate between NPC and healthy controls (Wei et al., 2008; Huang et al., 2009) and to differentiate between tumors that predominantly invade to the cranium versus tumors that would mainly manifest by extensive cervical lymph node metastasis (Guo et al., 2005). In addition, two separate studies have shown the existence of serum autoantibodies against NPC tumor proteins (Xiao et al., 2007; Tong et al., 2008). Studies comparing primary NPC tissues against normal epithelium shows that cathepsin D and stathmin are upregulated while 14-3-3σ, annexin I and SCCA1 are downregulated in NPC (Cheng et al., 2008; Yi et al., 2008; Xiao et al., 2010). Meanwhile, one laboratory has developed an online-accessible NPC proteome database from proteomic data generated from two-dimensional gel electrophoresis (Li et al., 2006; Li et al., 2007; Peng et al., 2009). Proteomic studies investigating differential protein expression of the stroma of NPC versus normal nasopharyngeal epithelial tissues revealed the differential protein level of CapG (Li et al., 2010), L-plastin as well as S100A9 (Li et al., 2009).

1.4 THE FOUR-JOINTED BOX ONE (FJX1) HUMAN GENE

The human FJX1 gene had no previous association with NPC. In this study, FJX1 was identified as the gene of interest as it was found to be upregulated in NPC, while expressed at limited amount in normal organs. While the exact function of FJX1 in human is unknown, subsequent information below describes current knowledge on FJX1 and its counterparts in other species. Also described is the amplification of 11p13, the region where FJX1 resides, in many human cancers.

1.4.1 Background

The human FJX1 was first identified via Expressed Sequence Tag (EST) clone isolation (Thate et al., 1995). The gene contains a single exon at chromosome 11p13 (Figure 1.2). The FJX1 protein is predicted to be a type II transmembrane glycoprotein, consisting of a short cytoplasm-facing C-terminus tail, a single transmembrane domain, followed by a predicted signal peptidase cleavage site, and a longer N-terminus region that could be facing inside of Golgi apparatus and/or the extracellular space (Figure 1.3). The protein shares 29% identity with its *Drosophila* equivalent Four-jointed (Fj) and 88% identity with mouse FJX1 (Rock et al., 2005).

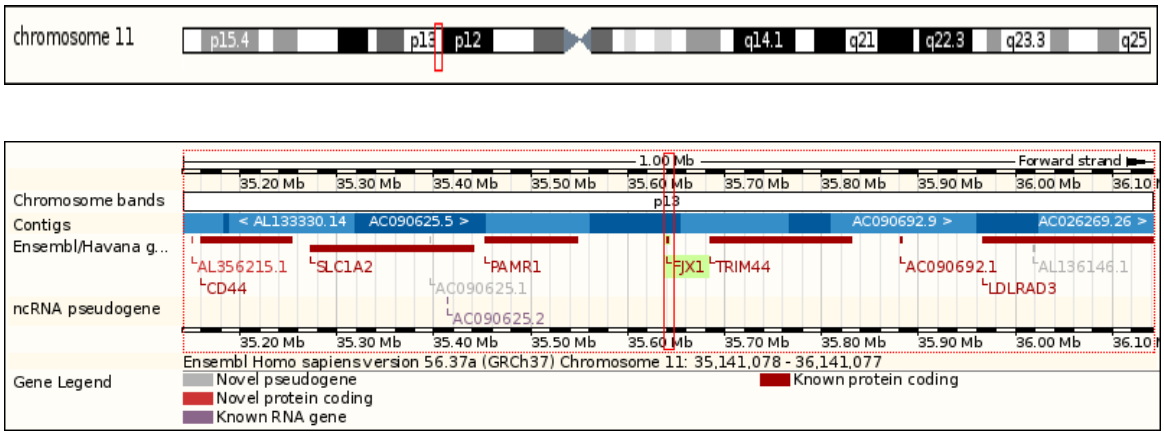

Figure 1.2| Chromosomal location of human FJX1. FJX1 is located at 11p13 chromosome. Image generated from Ensembl (http://www.ensembl.org/).

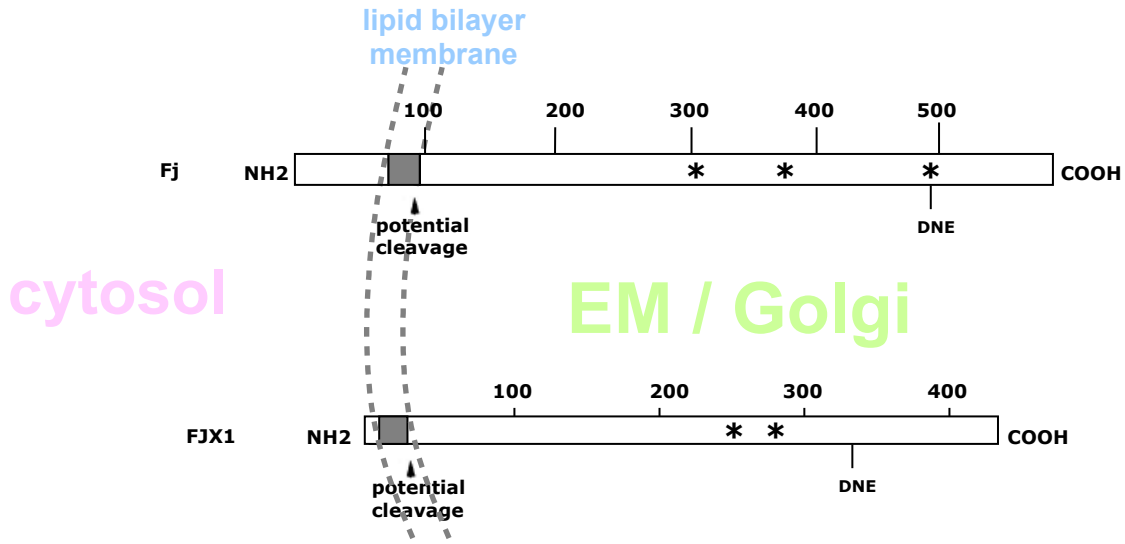

Figure 1.3| Human FJX1 protein. Human FJX1 is predicted to be a type II transmembrane glycoprotein, and shares 29% identity with *Drosophila* Fj (Villano & Katz, 1995; Rock et al., 2005), a Golgi-resident kinase which phosphorylates specific cadherins in two membrane-bound proteins Fat (Ft) and Dachsous (Ds) (Ishikawa et al., 2008). A previously documented crucial aspartic acid-asparigine-glutamine (DNE) motif for its kinase activity is conserved in human FJX1. Diagram of Fj is adapted from Villano & Katz (1995). Asterisks (*) denotes potential *N*-linked glycosylation sites. "EM" denotes the extracellular matrix space.

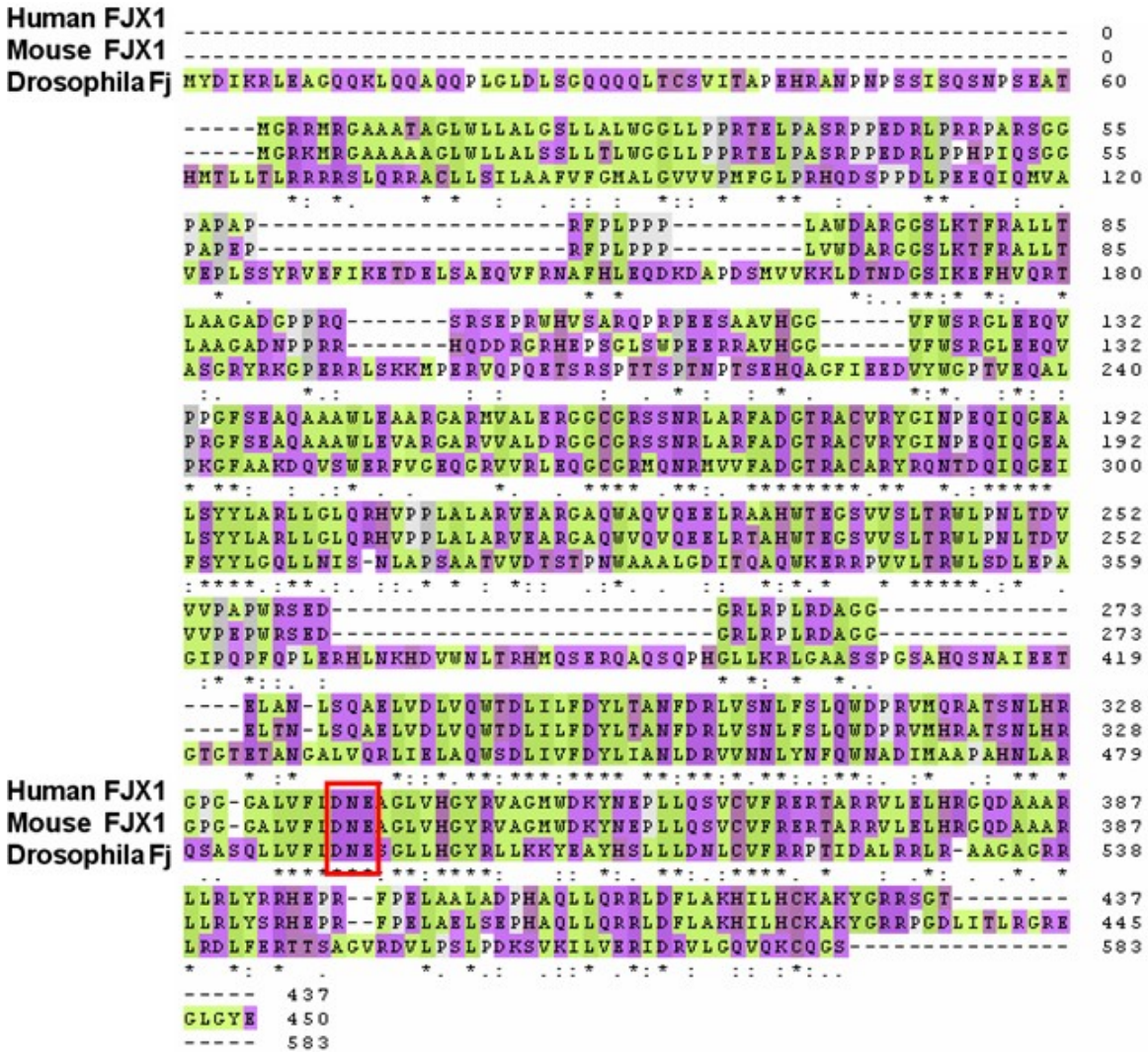

Figure 1.4| Protein sequence alignment of human and mouse FJX1 and *Drosophila* Fj. Red box denotes the conserved DNE motif crucial for kinase activity in Fj (Ishikawa et al., 2008). Highly hydrophobic neighboring residues are also conserved across the species (immediate left and right of the red box), suggestive of the conservation of the kinase activity.

1.4.2 Subcellular Localization

A proteomic study on proteins secreted by human ovarian cancer cell line ES2 reported that FJX1 can be found in culture medium (Faca et al., 2008). Its mouse counterpart has also been shown to be secreted into the medium (Rock et al., 2005). Addition of recombinant mouse FJX1 to the culture medium causes a decrease in dendrite extension in cultured mouse neurons - the same effect conferred by FJX1 overexpression from plasmid transfection to the cells itself (Probst et al., 2007). On the flipside, secretion of *Drosophila* Four-jointed is not crucial for its function, and its preferential localization in the Golgi is accompanied by an elevated functional activity (Strutt et al., 2004).

1.4.3 Molecular Function

The exact molecular function of human FJX1 is unknown. In *Drosophila*, Fj is a Golgi-resident kinase that phosphorylates specific cadherin domains in two gigantic transmembrane proteins Fat and Dachsous (Ishikawa et al., 2008) to regulate their heterodimeric interaction (Brittle et al., 2010; Simon et al., 2010). The aspartic acid-asparigine-glutamic acid (DNE) motif that is crucial for the kinase activity is conserved in human and mouse (Figures 1.3 and 1.4), as well as in other FJX1 proteins in other animals.

Clues in other species hint that human FJX1 could be a part of several biological pathways. The Fj, the *Drosophila* Fat and its murine orthologue Fat4 have been shown to control the Hippo tumor suppressor pathway that regulates organ size by regulating proliferation and apoptosis (Bao et al., 2011). The corruption of this pathway has been increasingly recognized in many different human cancers (Zhao et al., 2010). In mouse, either the loss of Fat4 or an increase in the activity of the pathway effector YAP is accompanied by increased FJX1 expression (Happe et al., 2011; Saburi et al., 2008). In addition, both

Drosophila Fj and murine FJX1 were shown to be involved in the Notch signaling pathway (Buckles et al., 2001; Rock et al., 2005). Furthermore, Gutierrez-Avino *et al*., has shown that Fj also mediates the function of JAK/STAT pathway, and that Fj might be a regulatory node that integrates multiple growth-controlling pathways in flies (Gutierrez-Avino et al., 2009).

Currently there is not much knowledge available on the status of the aforementioned pathways in NPC. A component of the Hippo pathway LATS2, largely known to function as tumor suppressors in other cancers, is overexpressed as a result of demethylation in NPC, and its expression is an indicator of poor prognosis (Zhang et al., 2010). Meanwhile, the Notch signaling pathway is predominantly activated in a subpopulation of NPC cells that co-express embryonic stem cell markers (Zhang et al., 2010).

1.4.4 Physiological Function

Various studies have pointed to possible involvement of FJX1 in animal development. *In situ* hybridization analyses have demonstrated specific FJX1 expression in early developing neuronal structures of chicken (Yamaguchi et al., 2006), mouse (Ashery-Padan et al., 1999) and zebrafish, where its expression is regulated by the epigenetic regulator Histone Deacetylase (HDAC1) (Harrison et al., 2011). FJX1 is also specifically expressed in neural but is absent in embryonic stem cells in mice (D'Amour & Gage, 2003). FJX1 is also important in the proper neuronal function as cultured neurons derived from FJX1 null mice were defective in forming dendrite branching, an important cellular structure for neuronal cell function, even though the knockout FJX1 mice were viable and fertile (Probst et al., 2007). Furthermore, the brain of adult mice also express very low FJX1 transcript (Ashery-Padan et al., 1999; Rock et al., 2005a; Rock et al., 2005b).

FJX1 might also be important in kidney physiology. Simultaneous loss of FJX1 and Fat4 synergistically worsens cystic defects in mouse kidneys (Saburi et al., 2008). FJX1 expression is also required for tissue regeneration in mouse kidney after injury (Happe et al., 2009). Furthermore, even though murine FJX1 is expressed in the kidneys of mouse embryo, it is no longer expressed in adult kidneys (Li et al., 2009). Together, this suggests that FJX1 might be important in the development and tissue repair of murine kidney.

Other clues hint strongly at FJX1 possible involvement in tissue proliferation and organ growth. FJX1 is expressed at the proliferation zone of the lens and the feather buds of developing chicken (Yamaguchi et al., 2006). It is also expressed during limb outgrowth in chick (Yamaguchi et al., 2006) and in mouse (Ashery-Padan et al., 1999). In addition, RNAi experiments targeting FJX1 during cricket leg regeneration resulted in slight leg shortening in the insects after leg amputation (Bando et al., 2009).

Other studies on FJX1 counterparts meanwhile point to their role in regulating planar cell polarity (the patterning of epithelial cells) in flies (Zeidler et al., 2000; Casal et al., 2002; Simon et al., 2004; Strutt et al., 2004; Segalen et al., 2009; Zhu et al., 2009) and in mouse (Saburi et al., 2008). Interestingly, in developing mouse kidney, lung and intestine, FJX1 is expressed by the epithelial cells that reside at the boundary of the mesenchymal-epithelial interaction in the organs (Rock et al., 2005a; Rock et al., 2005b). Collectively, these studies suggest that FJX1 proteins might be involved in the physiology of the nervous system and the kidneys, in proliferating cells during organ development and regeneration, as well as in planar cell polarity determination.

1.4.5 FJX1 in Neoplasia

1.4.5.1 Expression

FJX1 transcript is upregulated in ovarian tumor vasculature (Buckanovich et al., 2007; Lu et al., 2007). Buckanovich *et al*., also further reported that FJX1 is expressed in reproductive tissues with physiological angiogenesis (e.g. corpus luteum, endometrium, placenta) at almost similar level as in ovarian-tumor derived endothelial cells, suggesting that FJX1 might have a role in the process (Buckanovich et al., 2007). In addition, in mice injected with human small cell lung cancer cells, FJX1 was found to be predominantly expressed in metastatic cells in the kidneys (Kakiuchi et al., 2003). On the contrary, FJX1 was found to be downregulated in mycosis fungoides, a form of cutaneous T-cell lymphoma, when compared to inflamed dermal tissues (Tracey et al., 2003).

1.4.5.2 FJX1 and 11p13 Chromosome Amplification in Human Cancers

Human FJX1 is located in the 11p13 chromosomal region. Even though there has been no previous studies pointing to a structural aberration of the region in NPC, it was found to be amplified across many types of primary tumors, including the breasts (Klingbeil et al., 2010), lungs (Lockwood et al., 2008; Sung et al., 2010; Starczynowski et al., 2011), gastric (Carvalho et al., 2006; Zhang et al., 2011), oral (Snijders et al., 2005), esophageous (Miller et al., 2006; Chattopadhyay et al., 2010), prostate (Ma et al., 2009), small bowel (Diosdado et al., 2010) and acute myeloid myeloma (Sarova et al., 2010). In oral squamous cell carcinoma, FJX1 is both overexpressed and amplified in oral squamous cell carcinoma (Snijders et al., 2005).

Furthermore, other reports have shown that 11p13 is a frequent amplicon in cell lines derived from oral (Jarvinen et al., 2008), breast (Forozan et al., 1999), lung (Lockwood et al.,

2008; Sung et al., 2010; Starczynowski et al., 2011), and gastric (Fukuda et al., 2000) cancers. In addition, 11p13 is found to be significantly amplified in a huge study involving cancer cell lines of various origins (Beroukhim et al., 2010). In basal-like breast cancer, a neighboring gene CD44, was previously shown not to be the amplicon driver (Klingbeil et al., 2010), while TRAF6 was demonstrated to be one of the amplicon target in non-small-cell lung cancer (Starczynowski et al., 2011). Together, the results of these studies suggest that the amplification of 11p13 region might be important to the development of cancer in general, and FJX1 could be one of the amplicon targets.

1.5 HYPOTHESIS AND OBJECTIVES OF THE STUDY

Cancer Research Initiatives Foundation (CARIF) has conducted an expression microarray study to analyze genes that are differentially expressed in NPC relative to nonmalignant nasopharyngeal epithelial tissues from Malaysian subjects (Bose et al., 2009). With the previous identification of differentially upregulated genes in NPC from the study as the starting point, the hypothesis of this study is that genes that are significantly upregulated in NPC samples studied could be driver oncogenes in, or could serve as biomarkers for the disease.

The purpose of this study is to understand the molecular basis of NPC with an aim to develop biomarker(s) or therapeutic target(s) for NPC. The specific objectives were:

1. **To identify genes that were up-regulated in NPC, but expressed minimally in normal human organs using expression microarrays**
2. **To validate the microarray data by examining mRNA expression of the candidate genes in NPC tissue samples and normal human organs by quantitative- and semi-quantitative PCR, respectively.**

3. **To examine the protein expression of the candidate genes in primary NPC tissue samples by immunohistochemistry, and to correlate the protein expression with clinicopathological features**
4. **To determine the *in vitro* significance on alteration of the level of the candidate gene, which is FJX1, to the oncogenic traits of NPC cells**