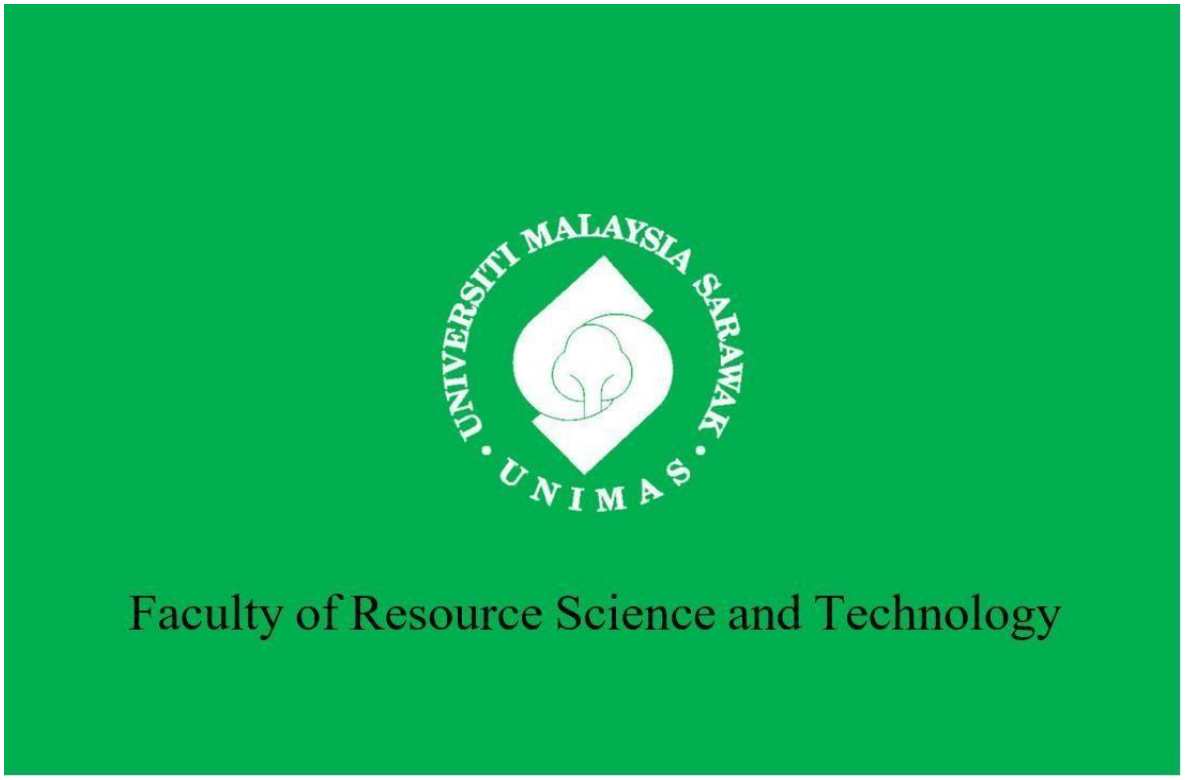

Bibliographic analysis of p53 gene family and gene expression analysis of p63 in Nasopharyngeal Carcinoma (NPC)

Thanes A/L Gunasekaran (71805)

Bachelor of Science with Honours
(Resource Biotechonology)
2022

Bibliographic analysis of P53 gene family and gene expression analysis of P63 in Nasopharyngeal Carcinoma (NPC)

Thanes A/L Gunasekaran

A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of Science with Honours
(Resource Biotechnology)

SUPERVISOR: PROFESSOR DR EDMUND SIM UI HANG

Programme of Resource Biotechnology
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UNIVERSITI MALAYSIA SARAWAK
2022

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BIBLIOGRAPHIC ANALYSIS OF P53 GENE FAMILY AND GENE EXPRESSION ANALYSIS OF P63 IN NASOPHARYNGEAL CARCINOMA(NPC)

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ABSTRACT

Nasopharyngeal Carcinoma (NPC) is one of the common diseases in Asia countries especially in Southern China. According to Malaysia National Cancer Registry (NCR) on the year 2002, its ranked NPC as the second and high rate of cases for males (8%) from all cancers for males in Malaysia. While for female it has been ranked as tenth cancer which was 2.8% of all cancer diagnosed by Malaysia females. Cancer has been proven to be associated directly or indirectly to gene. The Tumor protein gene *p63* might lead to the occurrence of carcinogenesis as well. There are still lack of evidence of the NPC predisposition gene that will development the tumor growth. Previous research has been suggested that *p63* gene have been involved in NPC development. In this study, gene expression analysis was conducted on *p63* with normal epithelium cells and tumor cell where it suggested that p63 gene maybe a possible factor for NPC predispositions. Besides that, this study also focuses on bibliographic analysis to discover the research trend on *p53* gene family in nasopharyngeal carcinoma and the institution that have been contributes to the research. In this study it concludes that *p63* gene is not differential expression in both cell lines which are tumor cell (TW04) and normal cell (NP69) and for the bibliographic analysis the number of publications on p*53* gene family in nasopharyngeal carcinoma have been recorded highest on the year 2014 while Central South University from China have been identified as the most contribute institution on this research.

Keywords: Nasopharyngeal carcinoma, *p63* gene, *P53* gene, gene expression, bibliographic

ABSTRAK

Karsinoma nasofaringel (NPC) merupakan sejenis penyakit yang biasa di negara Asia terutamanya, di Selatan China. Menurut Pendaftar Kanser Kebangsaan (NCR) Malaysia pada tahun 2002 telah mencatatkan tempat yang kedua dengan menyumbang 8 % daripada semua kanser kaum lelaki di Malaysia. Manakala, bagi kaum wanita kanser ini mencatat kedudukan tempat kesepuluh iaitu 2.8 peratus daripada semua jenis kanser rawatan Wanita Malaysia. Gen p63 mungkin mempunyai kecenderungan untuk sel kanser menumbuh. Pada masa kini, masih tidak ada gen berkecenderungan yang jelas dalam NPC. Namun demikian, penyiasatan sebelum ini menerangkan aktiviti gen *p63* terlibat dalam pengedaran kitaran sel dalam sel NPC. Pengekspresan gen analisis sedang dijalankan dalam kajian ini dalam sel normal dan sel tumor. Penyiasatan ini mencadangkan bahawa gen p63 merupakan satu faktor pengedaran kitaran sel dalam sel NPC. Selain itu, penyiasatan ini juga memberi fokus kepada analisis bibliografi tentang gen *p53* dalam NPC untuk mengaji trend penyelidikan dan insitusi yang menyumbang kepada penyelidikan karsinoma nasofaringel (NPC). Penyiasatan ini tidak menunjukan bahawa sel normal dan sel tumor mempunyai pengeksperesan yang berlainan. Bagi analisi bibliografi, bilangan penerbitan artikel tertinggi berkaitan p53 gen dalam NPC dicatatkan pada tahun 2014 manakala Central South University dari negara China memberi sumbanga tertinggi untuk pernyiasatan ini.

Kata Kunci: karsinoma nasofaringel, gen p*63*, gen p*53*, Pengekspresan gen, analisis bibliografi

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List of Abbreviations

AGE	Agarose Gel Electrophoresis
BLAST	Basic Local Alignment Search Tool
cDNA	Complementary Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
M MLV-RT	Moloney Murine Leukemia Virus Reverse Transcriptase
NCBI	National Centre for Biotechnology Information
NPC	Nasopharyngeal Cancer
mRNA	Messenger Ribonucleic Acid
PCR	Polymerase Chain Reaction
TP53	Tumor Protein p53 gene
TP63	Tumor Protein p63 gene
RPs	Ribosomal Proteins
rRNA	Ribosomal Ribonucleic Acid

CHAPTHER 1

INTRODUCTION

1.1 Study Background

The development of cancer at the nasopharynx, the region of the pharynx superior to the soft plate is refer as nasopharyngeal carcinoma or NPC. It known as the most common malignant tumor of nasopharynx which is consist around 0.7% of cancer in worldwide. Nasopharyngeal carcinoma (NPC) is highly prevalent in most Southeast Asia countries like Southern China, Hong Kong, Malaysia, Singapore, and Taiwan compared to western and middle east countries (Chen et al.,2009). It was estimated that man is likely develop NPC cancer two or three times more compared to women. Nasopharyngeal cancer is ranked first in Malaysia especially among the males who have in the age group between 15-49 which bring the percentage 17.7% (Kumar et al.,2003)

It also ranked as second among the top ten frequent cancers that diagnosed among Malaysia men with 8.8 percentage which relatively compared with lung, colon, leukemia, rectum, prostate, skin, liver, stomach, and lymphomas cancers. Nasopharyngeal carcinoma (NPC) among females is ranked in twelfth which was 2.5 percent when compared to all the cancers that diagnosed in Malaysia females. According to the Report for the National Cancer Registry 2003, there are around 1125 cases of nasopharyngeal cancer was recorded among 100000 population of males and females. The ratio of NPC occurrence between male to female is 2.75:1 which indicated that males carry higher incidence when compared to female (National Cancer Registry Malaysia (NCR),2002)

World health organization (WHO) have recognized the three subtypes of nasopharyngeal carcinoma (NPC) which are WHO type 1: Keratinizing squamous cell carcinoma, WHO type 2: Nonkeratinizing squamous cell carcinoma and WHO type 3: Undifferentiated carcinoma (Hoe et al.,2006). There are mainly

three factors contribute to the development of NPC at primary level which are genetic factors like gene expression or mutation, Epstein-Barr virus, and external environmental factors such as diet and consumptions. The genetic abnormalities at human body can trigger the nasopharyngeal carcinoma occur and it stimulates the tumorigenesis pathways of NPC.

Bibliographic analysis is one of the quantitative methods that approaches the analysis and measure the productivity like the number of publications to help researchers understand the growth and impact of scientific literature. There are a lot of online database software such as Scopus, PubMed and Web of science will help to collect the databases and analyzed it. Although there are a lot of scientific literature regarding NPC is doneby researchers, the articles that related to the *p53* gene family in nasopharyngeal carcinoma is limited. This is because only few articles have been analyzed the high cited NPC papers regarding the *p53* gene in NPC (Lo et al.,2002). Therefore, in this study we aimed to identify the top 100 most cited NPC articles that giving information regarding the *p53* gene in NPC from 2000-2022 using bibliographic analysis. This will help to comprehensively increase the understanding about the NPC development trend and research focus.

p53 gene is known as tumor suppressor gene which highly mutated around 50% of human cancers but it rarely mutates in nasopharyngeal carcinoma (NPC). The *p63* gene is one of the members of *p53* family where it has similar structure and function with other member of *p53* gene family including *p73*. *P63* will gives dominant negative effect on the function of p53 gene, and it function as one the role in the pathogenesis of nasopharyngeal carcinoma. The role of *p53* gene in NPC cancer which regulates the cell cycle and apoptosis is studied and well document. Over the pass decades, there are a lot of scientific research about the nasopharyngeal carcinoma take places to addressing the burden of diseases by improving healthcare and making the result available to wider scientific knowledge about the NPC cancer (Laherty et al., 1992).

Besides that, gene expression can be one of the factors that contribute for trigger the primary NPC occurrence and its also will affect the NPC pathogenesis pathways (Lo and Huang,2002). Previously there are several gene expression studies have been conducted for constructing the gene expression profile for nasopharyngeal carcinoma. However, the data produced by previous research from gene expression in NPC is still not sufficient regarding the expression of *p63* gene to completely to study the nasopharyngeal carcinoma tumorigenesis pathway. Gene expression analysis studies on NPC are needed to solve this problem. The hypothesis of the research is aimed to study the gene expression of *p63* gene in NPC by comparing the normal cell line (NP69)and cancerous cell line (TW04).

1.1. Objectives

The objectives of this study are

1.1.1. To conduct bibliographic analysis on *P53* gene family in nasopharyngeal carcinoma from 2000 to 2022 for study the research trend and the institution that made contribution.

1.1.2. To compare the gene expression analysis of *p63* gene in nasopharyngeal carcinoma by comparing the tumor cell (TW04) and normal cell (NP69).

CHAPTER 2

LITERATURE REVIEW

2.1. Nasopharyngeal

2.1.1 Anatomy of Nasopharyngeal.

The pharynx is divided into three parts which are nasopharynx, oropharynx, and laryngopharynx. The upper portion of the pharynx which specifically related to the respiratory system is known as nasopharynx. The portion of the pharynx to the soft plate where it connects the nose to the back of oropharynx (mouth) which helps to allowing the breathing through the nose and swallowing of mucus which produced by the membranes of the nose. There are two posterior opening of nasal chambers which lead into the single space of nasopharynx. The eustachian tubes or known as auditory tube also open into the nasopharynx. The auditory tubes is connect the nasopharynx with the middle ears. The pharyngeal tonsils known as lying in the upper posterior wall of nasopharynx. The soft palate floor of the nasopharynx is a trapdoor which closes off the upper respiratory passageways during swallowing (Deng et al,2010)

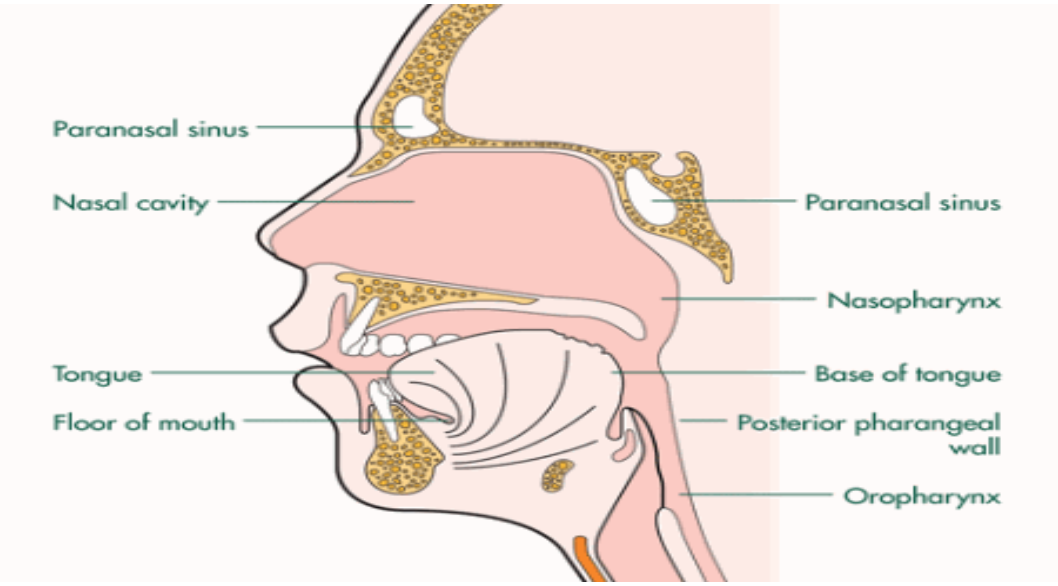

Figure 1: The anatomy of nasopharynx. Nasopharynx is located at the back of the entrance into the nasal passages (Adapted from htpp://www.cancerbackuo.org.uk/Cancertype/Headneck/Typesofheadneckcancers/Cancerofthenasopharynx,2005)

2.2. Nasopharyngeal Carcinoma (NPC)

Nasopharyngeal Carcinoma or NPC is known as development of malignancy in the nasopharynx. There are two types of tumours which can develop in the nasopharynx is known as benign tumor and malignant tumor. Benign nasopharyngeal tumour is known non-cancerous tumor formation where it normally found in the vascular system like angiofibromas and hemangiomas. Benign tumours are very rare occur in humans and it not spread to other part of body till it become cancerous tissue. While malignant tumour is ability to penetrate into the surrounding tissue and organs in the body. The development of tumour in nasopharynx from epithelial cells will cover the surface lining. Nasopharyngeal carcinoma (NPC) is one of rare malignancy is highly prevalent in Southern China with incidence rates between 25 and 50 per 100000 population. NPC is also known as "Canton Tumour" which derived from modern Chinese which means Guangdong. Eustachian tube in the Rosenmuller's fossa is the most common place nasopharyngeal carcinoma cancer occurs.

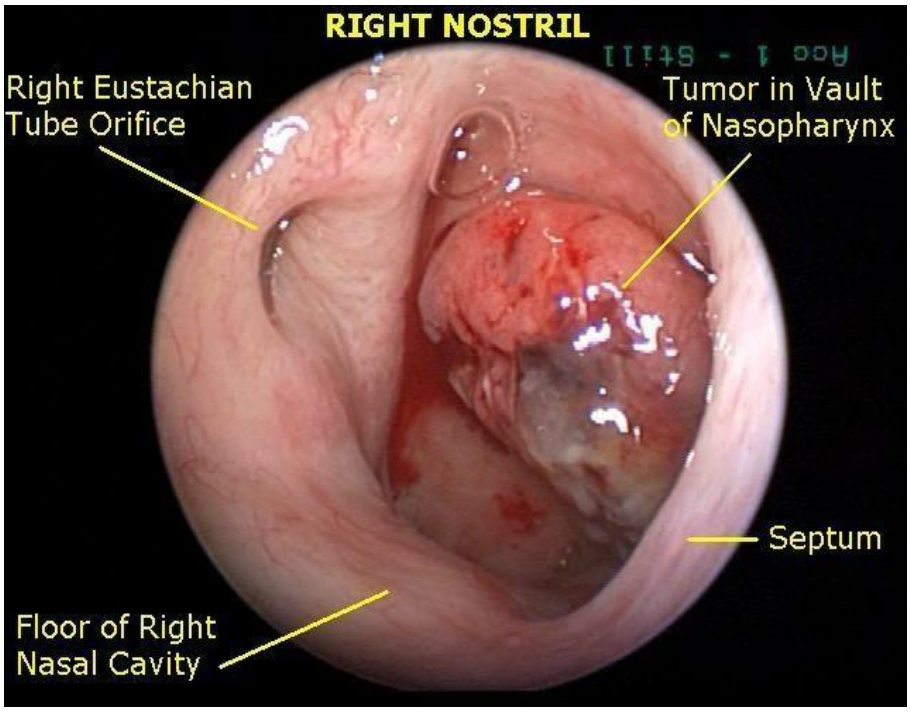

Figure 2: Tumour of nasopharynx. This picture depicts the right nasal endoscopy showing the nasopharyngeal tumour in nasopharynx (Taken from http://www.ghorayeb.com/Nasopharyngeal SquamousCellCarcinoma.html.2006)

2.2.1. Epidemiology of Nasopharyngeal Carcinoma (NPC)

According to GLOBAN 2018 statistic Nasopharyngeal Carcinoma (NPC) have been reported around 129000 cases where it brings around 0.7% of all the cancers. Countries like North America and Europe have been reported 1 case per 100000 populations. In Asia countries there are around 80 % of NPC cases where Southern China record high incidence rate (Vokes et al.,1997). According to Malaysia National Cancer Registry Report 2007-2011 among different race, Chinese have high rate of cases while in East Malaysia, Bidayuh have been report high incidence rate. According to the statistic Malaysia Chinese have been ranked high cases compared to Singapore Chinese. In figure 4 its shown that, Nasopharyngeal carcinoma have been recorded around 4.9% from the ten most common cancers in Malaysia.

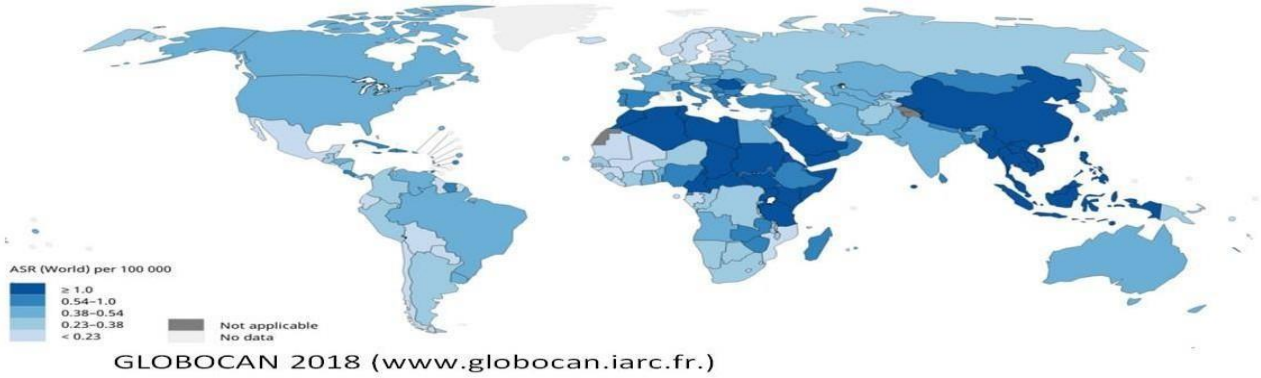

Figure 3: Map view on the geographical incidence of nasopharyngeal cancer across the globe in 2018.

(Adapted from http://www.npcresearch.org/)

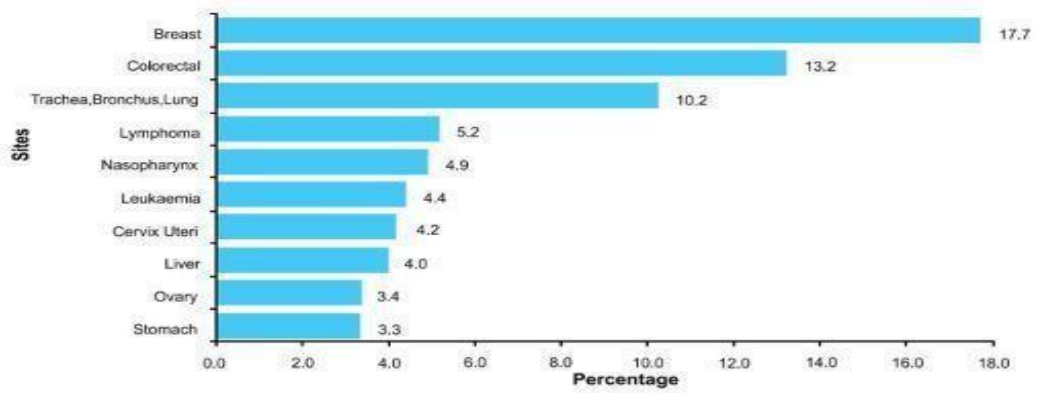

Figure 4: Percentage of ten most common cancers, Malaysia,2007-2011. (Adapted from http://www.npcresearch.org/)

2.2.2. Histopathology of Nasopharyngeal Carcinoma (NPC)

According to World Health Organization (WHO), there are three subtypes of nasopharyngeal carcinoma which are WHO type 1, WHO type 2 and WHO type 3. WHO type 1 known as keratinizing squamous cell carcinoma like carcinomas that arise from other sites of the head and neck (Fang et al.,2001). According to the study conducted by Krishna et al., (2004) the WHO type 1 tumours are less associated with EBV infection. The WHO type II is non-keratinizing squamous carcinoma where its strong association with Epstein-Barr virus infection. Although there is no microscopic evidence the cells will replicated. While WHO Type III will represents undifferentiated carcinomas which known as lymphoepithelioma (Vokes et al,1997). The WHO Type III is the most common form of NPC cancer and it has the strongest association with Epstein Barr virus (EBV) compared to type I and type II. (Sternberg,1999).

2.2.3. Genetic Susceptibility Nasopharyngeal Carcinoma (NPC)

Genetic susceptibility is the major risk factors that have been involves in the development of malignant tumor of nasopharyngeal. Genetic events play role in the development of nasopharyngeal cancer same as other types of human cancer. Genetic alterations are known as one of the features that help in develop human malignancies. The mutation and amplification in genes cause the increases of nasopharyngeal carcinoma. Hanahan and Weinberg have been termed this ‘Hallmarks of cancer where the genetic changes and malignant growth have been altering the cell physiology (Hanahan & Weinerg,2000). Metastasis, evasion of apoptosis, angiogenesis and tissue invasion will develop the NPC cancer. The number of genes that affect tumour cell behavior will involve in the process to achieve the cancer hallmark. The genes involve in tumor development always divided into two main groups which are tumor suppressor genes and oncogenes. The gene that contributes in the production of cancer by activating cellular proliferation, leading to unregulated cell growth and differentiation

is defined as an oncogene. While tumor suppressor gene are defined as the normal cellular gene with the function to inhibit the cellular proliferation and they are usually inactivated by 'loss of function' mutation in malignant tumour development. (Weinberg,1991). The "two hit" hypothesis which is proposed by Knudson in 1997 have been discovered the carcinogens or the cancer were caused by two mutational events. Tumor suppressor gene will inactivate either when a germline mutation or somatic inactivation which frequently detected in human DNA and at region of chromosomal loss for example region of Loss of Heterozygosity (LOH) (Kumar et al.2003).

2.3. Gene of study

The two genes targeted in these studies are tumor protein p53 gene and tumor protein p63 gene where both genes are located at 17p 13.1 and 3q28 respectively. Both gene is proposed to be involved in the stages of tumorigenesis in Nasopharyngeal Carcinoma (NPC).

2.3.1. Tumour Protein P53 gene

The tumour protein *p53* gene is one of the tumour suppressor gene which highly mutated in human which have caused the development of NPC cancer. *TP53* gene have been located at human short arm chromosome 17 and its transcription factor will control the cell cycle. It will cause the damage towards the cells at G1 checkpoint, and it will keep the gene cause apoptosis and it will repair. The *p53* gene will mutated and tumor cells maybe inactivated with it interact with viral proteins. The mutation of *p53* gene have been created an association with aggressive tumours (Sternberg,1999). The higher the frequency of mutations in the *p53* gene the higher the carcinoma resistant to chemotherapy, radiation, and anticancer drug. The mutation rate of *p53* gene is lower in nasopharyngeal carcinoma (NPC) compared to other cancer (Cao et al.,2008). Point mutation will be the most common mutation that associated in NPC (Fu et al.,2009). However, in this study we focus on bibliographic analysis of *P53* gene family in nasopharyngeal carcinoma to evaluate the research trend and affiliation on NPC.

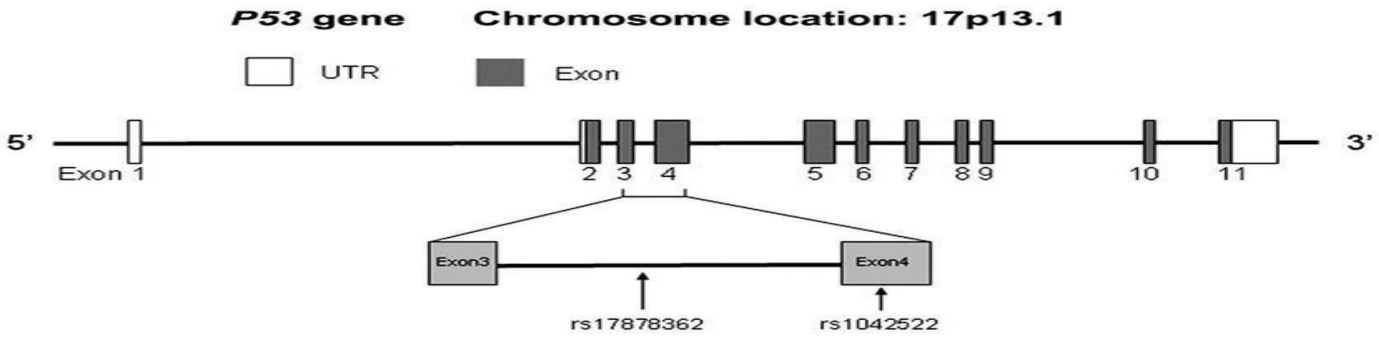

Figure 4: The structure of *P53* gene family. (Adapted from: doi: 10.1371/journal.pone.0061662.g001)

2.3.2. Tumor Protein *P63* gene

Tumor Protein *P63* gene is known as one of tumor suppressor gene or oncogenes under *p53* gene family where it contains multiple isoforms with various activities. There are three things contains in *p53* gene which are transactivation domain which have transcriptional activity and transactivate *p53* target genes. The multiple isoforms will play role in detecting the expression of *p63* and it determine in various tissues and in tumors (Fuet al.,2009). The structure of tumor protein *p63* gene is similar with tumor protein *p53* gene where the *p63* genewill also act as sensor for the DNA damage, induce the apoptosis and it's upregulated the cells with DNA damaging agents. The expression of *p63* was general detected in nasopharyngeal epithelial cells at their proliferation stage where its conclude that *p63* gene have been involved in tumorigenesis of nasopharyngeal carcinoma (Tang et al.,2012)

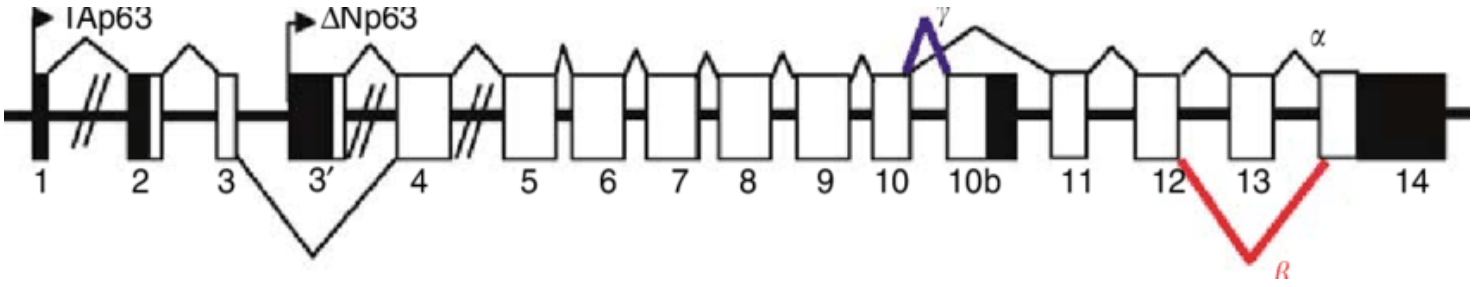

Figure 5: The structure of p63 gene family. (Adapted from: https://www.researchgate.net/publication/6200243_P53_and_its_isoforms_in_cancer#:~:text=3)%3A277%2? D82-,DOI%3A10.1038/sj.bjc.6603886,-SourcePubMed

2.3.3. Tumor Protein *P73* gene

Tumor Protein *P73* gene is one of the members under p53 gene family where it shares the similar structure and the cellular activities. P73 also one of the tumor suppressor proteins where it will undergo apoptosis and cell cycle arrest and it help in activates the p53 gene. The unique role of p53 gene is it will involve in differentiation and also in neuronal development. Apart form this, p73 gene also will involves in metabolic control and spermatogenesis for the maintance of male fertility. The previous research on the p73 gene shows that p73 is rarely mutated in human cancer like p53 gene, The function of p53 gene will overlap with p73 gene because it will undergo pro apoptotic roles of p53 gene in many cancers that it mutated. There are two isoforms under p73 gene which are Tap73 and DNp73. The stability and activity of p73 are primarily regulated by post translational modification (PTMs).

2.4. Pathogenesis of Nasopharyngeal Carcinoma

Previous research has been suggested that the development of Nasopharyngeal Carcinoma it is depends on the genetic events. The pathogenesis of NPC is directly involving the early events for NPC tumorigenesis. According to (Lo and Huang,2002) research it stated that initiated cells with allelic loss on 3p and 9p may have high chance in the growth of tumor and expand the form multiple clonal cells. The genetic factors like deletion on 14 q will also accumulated the genes and will cause the phenotypic and morphological changes will lead the tumorigenesis of nasopharyngeal carcinoma. The multiple genetic changes such as deletions of 11q,13q 16q and inactivation of p53 gene or p63 genes may be involved in the later steps during the development. (Jia et al.,2003). Several studies have been indicating the high rate of loss of heterozygosity (LOH) in NPC in chromosomes 3p,9p,9q,11q,12q,13q,14q,16q, and 17p suggesting the involvement of multiple tumor suppressor genes which will increase the pathogenesis of NPC (Tang et al.,2012).

2.5. Gene expression of Nasopharyngeal Carcinoma

Gene expression of *p63* gene was conducted by the researchers where it further investigates the relationship between the *p63* expression at differential stage of nasopharyngeal carcinoma. There are around 106 Nasopharyngeal carcinoma tissue sample have been included in the research where its containing 50 undifferentiated carcinoma,48 differentiated carcinomas and 8 keratinizing squamous cell carcinomas (Lo et al.,1995). The gene expression has been shown in differential stage NPC where all the tissue sample have been malignant. The gene expression of Tumor Protein *p63* gene have been indicates that there is possibility for *TP63* to be biomarker of Nasopharyngeal Carcinoma (NPC) (Lo et al.,1995).

CHAPTHER 3

MATERIALS AND METHODOLOGY

3.1. Methodology (Bibliographic analysis of *P53* gene family in NPC cancer)

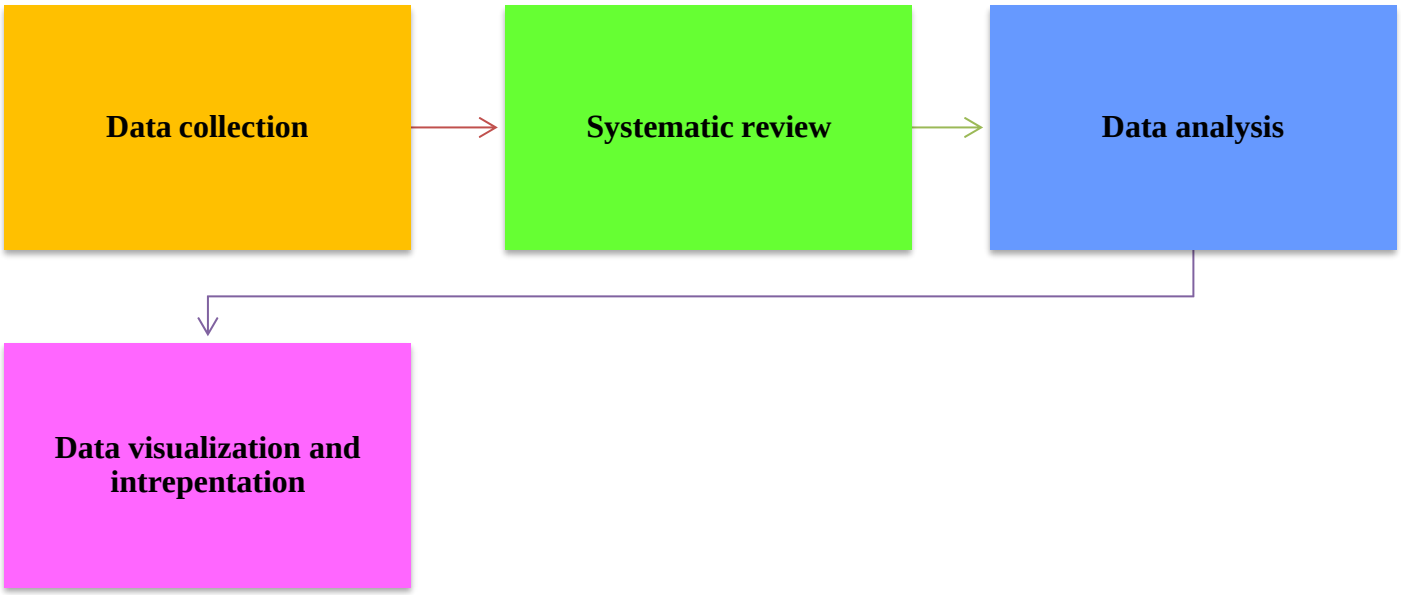

Figure 7: The flowchart of bibliographic analysis

3.1.1 Data collection

Online database software Scopus was used widely in this research to collect the data. The search strategy is important for this study to performed or to identify the terms of the research. The search process for this study carried out from the year 2000 until 2022. The objective of this study was to be conduct bibliographic analysisby using the following variable which are total number of publications, year of publication and affiliation or institutions. The keyword for the research is inserted which are “Nasopharyngeal carcinoma” and “p53 gene family in Nasopharyngeal carcinoma” where this is used as query string for collect the databases. Based on the suggested time period the publications associated with the study were discovered. The number of publications, year of publication and affiliation of publications were then retrieved.