



Faculty of Resource Science and Technology

**Bibliographic Analysis of p53-Gene Family and Gene Expression
Analysis of p73 in Nasopharyngeal Carcinoma**

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Bachelor of Science with Honours
(Resource Biotechnonology)
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**Bibliographic Analysis of p53-Gene Family and Gene Expression
Analysis of p73 in Nasopharyngeal Carcinoma**

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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
Faculty of Resource Science and Technology
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Bibliographic Analysis of p53-Gene Family and Gene Expression Analysis of p73 in Nasopharyngeal Carcinoma

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ABSTRACT

Nasopharyngeal Carcinoma (NPC) appears to be the most prevalent cancer specifically in East and Southeast Asia. NPC is a rare tumor occurs in the nasopharynx, often perceived at the pharyngeal recess or also known as fossa of Rosenmüller. p73 is known as the member of p53 family gene due to their allied in gene structure. Study suggests that the polymorphism of p73 responsible in mediating the nasopharyngeal carcinoma among Chinese populations. However, there is still lack of characterization between p73 gene and nasopharyngeal carcinoma. Apart from that, evidence showed that ribosomal proteins were vital as a tumor suppressor, gene-specific translational regulators, and oncogene. Therefore, the main purpose of this research is to perform a bibliographic analysis of the p53 in nasopharyngeal carcinoma publications and to compare the expression p73 gene in NPC cell lines by RT-PCR. PCR result revealed that the expected product size of p73 gene was 994bp. Smear bands were obtained on the p73 in both NP69, and SUNE-1 cell lines. Possibilities of the smeared result of p73 was discussed in this study.

Keywords: p53, Bibliographic analysis, p73, Gene expression, Nasopharyngeal Carcinoma

ABSTRAK

Karsinoma Nasofaring (NPC) muncul sebagai salah satu kanser yang berleluasa di Asia Tenggara. NPC merupakan tumor yang tumbuh di dalam nasofaring, yang juga dikenali sebagai ceruk pharyngeal atau juga dipanggil sebagai fossa rosenmiller. P73 dikenali sebagai ahli keluarga gen p53 disebabkan persekutuan dari segi struktur gen. Kajian mencadangkan bahawa polimorfisme p73 bertanggungjawab dalam pengantaraan karsinoma nasofaring dalam kalangan populasi Cina. Walau bagaimanapun, masih terdapat kekurangan pencirian antara gen p73 dan karsinoma nasofaring. Selain itu, bukti menunjukkan bahawa protein ribosom adalah penting sebagai penindas tumor, pengawal selia translasi khusus gen, dan onkogen. Oleh itu, tujuan utama penyelidikan ini adalah untuk melakukan analisis bibliografi p53 dalam penerbitan karsinoma nasofaring dan membandingkan ekspresi gen p73 dalam garisan sel NPC oleh RT-PCR. Keputusan PCR mendedahkan bahawa saiz produk jangkaan gen p73 ialah 994bp. Jalur berlumur diperolehi pada p73 dalam kedua-dua saluran sel NP69, dan SUNE-1. Kemungkinan hasil calitan p73 telah dibincangkan dalam kajian ini.

Kata kunci: p53, bibliografi analisis, p73, ekspresi gen, karsinoma nasofaring

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List of Abbreviation

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
NAD ⁺	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide + Hydrogen
NPC	Nasopharyngeal Carcinoma
mRNA	Messenger Ribonucleic Acid
PCR	Polymerase Chain Reaction
P73	Protein 73
RT-PCR	Reverse-Transcriptase Polymerase Chain Reaction

CHAPTER 1: INTRODUCTION

Nasopharyngeal Carcinoma (NPC) appears to be a major public health issue adequate to their increment in incidence and death rate (Jicman et al., 2021). NPC is commonly occurred among the Southern Chinese and rare among the western countries. The etiology of NPC is proven to be highly associated with Epstein-Barr virus (EBV) (Guo et al., 2006). NPC vary from the other head and neck cancers in terms of its histopathologic features, response to therapy, epidemiologic features, and treatment strategies (Chang & Adami, 2006). According to the World Health Organization (WHO) regarding NPC's histopathology classification, NPCs are classified into non-keratinizing carcinoma and keratinizing squamous cell carcinoma (KSCC) (Wang et al., 2016).

A 373 amino acid nuclear phosphoprotein, p53 tumor suppressor gene mutated in most of the human cancers, which is about 90%, but it rarely undergoes mutation in NPC (Guo et al., 2006). The transcription factor of the p53 family gene comprises three proteins, which are p53, p63, and p73 (Rozenberg et al., 2021). In opposition, p73 is expressed in several malignancies including breast cancer (Dominguez et al., 2001), lung cancer, stomach, and esophagus adenocarcinomas ((Tomkova, Belkhiri, El-Rifai, & Zaika, 2004), and other solid tumors. Zhang et al. suggest that the polymorphism of p73 responsible in mediating the nasopharyngeal carcinoma among Chinese populations.

Previous studies had shown the relationship between the induction of p53 signaling pathway that encourages the occurrence of extensive apoptosis in progenitor cell types, which leads to ribosomopathies, despite the elusive relationship of these ribosomal diseases and the high incidence malignancy rate in patients. To further examine the publications trend of p53 and the

expression of p73 in nasopharyngeal carcinoma, a bibliographic analysis and gene expression of p73 in nasopharyngeal carcinoma were performed.

In this project, a bibliographic analysis on the publications of p53 family gene in nasopharyngeal carcinoma was carried out to study the plot trend of annual scientific production, country scientific production, affiliations, and the corresponding author's country from year 2000 until 2022 via a software known as RStudio version 4.2. The bibliographic study allows the systematic review of the evolutionary nuances of the p53 family gene publications and studies, specifically in nasopharynx cancer field of study.

On the other hand, Zhang et al. (2014) suggest that the polymorphism of p73 responsible in mediating the nasopharyngeal carcinoma among Chinese populations. To study the regulation of the p73 gene in nasopharyngeal carcinoma, a reverse transcription polymerase chain reaction (RT-PCR) was conducted in the immunology genetic laboratory UNIMAS. A local normal NP69 cell line and a tumor SUNE-1 NPC cell line were utilized to verify the expression of p73 in NPC.

Hence, the objectives of this research were:

1. To investigate the publications plot trend of p53 in nasopharyngeal carcinoma research published from year 2000-2022 via Scopus database.
2. To identify and quantify the expression of gene p73 in NPC cell line compared to normal NPC cell line.

CHAPTER 2: LITERATURE REVIEW

2.1 Nasopharyngeal Carcinoma

Nasopharynx cancer is a highly macabre disorder due to the poor survival rate (Jeyakumar et al., 2006). Nasopharyngeal carcinoma accounts for more than 95% in adults and 35% in children (Jeaykumar et al., 2006). The early diagnosis of nasopharyngeal often misgauge owing to the uncertain presenting symptoms and the strenuous nasopharynx malignant examination. Nasopharyngeal carcinoma however is relatively uncommon. According to Chen et al. (2019), there were only 0.7% nasopharyngeal cases accounted of all cancers diagnosed in 2018, and 70% of the new cases were occurred Southeast Asia (Wu et al., 2018).

The symptoms in presenting the occurrence of NPC include epistaxis, cephalalgia, nasal obstruction and rhinorrhea, and any other nonspecific indicators. There are several initiatives of treatment for nasopharynx cancer, such as radiotherapy, surgery, chemotherapy, and chemoradiation (Sucipto et al., 2019). Nasopharyngeal anatomy is the factor the surgery initiative is rarely performed, which is located behind the rhinal (Sucipto et al., 2019). Endoscopic biopsy and examination were established as the analytical system for diagnosing nasopharyngeal carcinoma (King et al., 2019). NPCs that unable to be seen from the endoscopic view were usually discovered either in the pharyngeal roof or recess, and still in the early-stage of cancers, which are still in the higher rates of survival as compared to advanced-stage cancers (Lee et al., 2014).

Figure 1 shows the magnetic resonance imaging (MRI) result of the expanding nasopharyngeal carcinoma endoscopy of a 42-year-old man. A small nasopharyngeal was detected in the right side of pharyngeal recess, slowly growing over the 36 months period. (a) Showed the initial endoscopic examination. Small tumor was detected on the MRI scan (ai) but undetectable on

the endoscopic examination (aii). (bi) The tumor increased slightly in size after 30.3 months. (bii) no sign of tumor presence. (ci) white arrow shows the extending outside the recess showed on MRI. (cii) A small tumor (white arrow) detected on endoscopic examination after 36.3 months after the first examination (King et al., 2019).

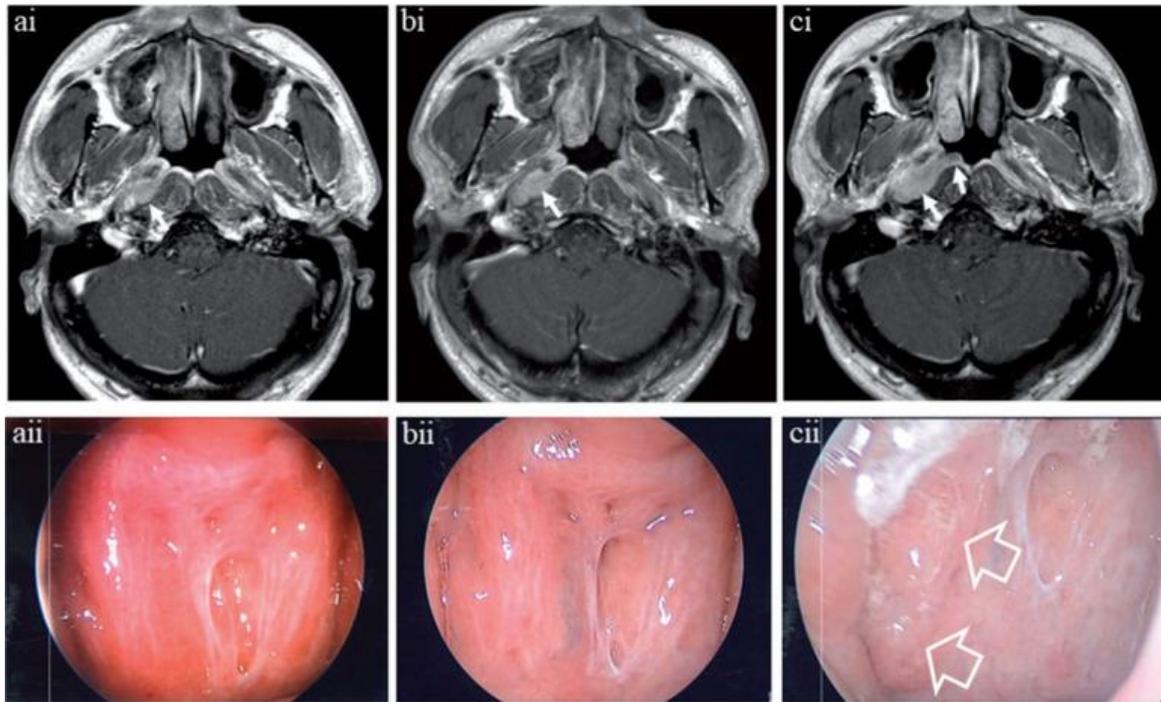


Figure 1: A growth pattern analysis of a patient's nasopharyngeal carcinoma in the right side of pharyngeal recess (King et al, 2019).

Nasopharyngeal carcinoma is prone to be detected when it reaches at advanced stages: Stage III and IV when diagnosed (Wu et al., 2018). The examination of NPC is initially utilized an indirect nasopharyngoscope, followed by fiberoptic endoscope. The utilization of advanced imaging procedures such as MRI or CT scan are preferred when the suspected tumor is not visible under endoscopic examination (Wu et al, 2019).

2.1.1 Etiological Factors of Nasopharyngeal Carcinoma

According to World Health Organization (WHO), the pathological subtypes of nasopharyngeal carcinoma can be classified into three: non-keratinizing, keratinizing squamous, and basaloid squamous. The keratinizing subtype occurred relatively rare in endemic areas such as southern China, while the non-keratinizing NPC subtype account for most cases in endemic areas by over 95% and is predominantly correlated with EBV (Epstein-Barr virus) infection (Young & Dawson, 2014; Wang et al., 2016). The development of NPC has spurred studies on its risk factor, including the environmental factors, EBV infection and genetic susceptibility.

2.1.1.1 Environmental Factors

There are some noticeable features on the population with higher risk of NPC, including Cantonese ethnicity, which is high consumption of salted preserved fish (Jeannel et al., 1993). Most studied suggests that the over-consumption of salted preserved fish during all time periods has a very strong correlation with nasopharyngeal carcinoma (Yu et al., 1986). Salted fish was found to contain carcinogenic and EBV-stimulating substances.

In addition, people with insufficient of fruits and vegetables consumption tends to be exposed to NPC risk than people with balanced diet. The epidemiological studies indicates that the long-term exposure to environment with toxic pollutants linked to elevate the NPC incidence (Armstrong et al., 2000; Li et al., 2006). Besides, a long-term cigarette smoker and alcohol drinker will have higher chance in getting NPC.

2.1.1.2 Genetic Susceptibility

Among the Chinese populations, genetic predispositions contribute to the development of NPC as suggest by the studies. According to Lu et al. (2003;2005), genes within the HLA-A locus are associated with susceptibility to nasopharyngeal carcinoma. In recent years, chromosomes 3p21, 4 and 5p13 were identified as the putative genetic susceptibility loci in NPC (Zeng et al., 2006; Hu et al., 2008). However, the candidate of NPC genes has not been determined due to the large chromosomal regions.

CYP2E1 is strongly associated with the NPC susceptibility. It is responsible in the metabolic activation of an EBV activating agent, known as nitrosamines and other carcinogens. GTSM1 known for its roles in carcinogens detoxification, and its absence will lead to the increased risk of nasopharyngeal carcinoma (Nazar-Stewart et al., 1999). Individual with inherited nasopharynx cancer tends to have increased risk of developing NPC.

2.2 Cell Lines of Nasopharyngeal Carcinoma

Cell lines generally used as primary cells to examine the biological pathway. Cell lines provides unlimited material supply and neglect the ethical concerns related to the utilization of human and animal tissues (Kaur & Dufour, 2012). Cell lines yield a pure cells population, which provides a consistent and reproducible outcome. Cell lines are widely used in scientific research and to study the gene function and expression such as the gene expression of p73 gene in nasopharyngeal carcinoma.

2.2.1 NP69

NP69 (Figure 2) is a human nasopharyngeal epithelial cell line that retains various traits of normal nasopharyngeal cells, including the profile of the keratin and feedback to transforming growth factor beta (TGF- β) (“NP69 Human Nasopharyngeal Epithelial Cell Line”, 2016). NP69 cell lines are non-tumorigenic cells and demonstrate the growth of an anchorage dependent cell. It is essential in studying the viral-associated tumorigenesis mechanisms.

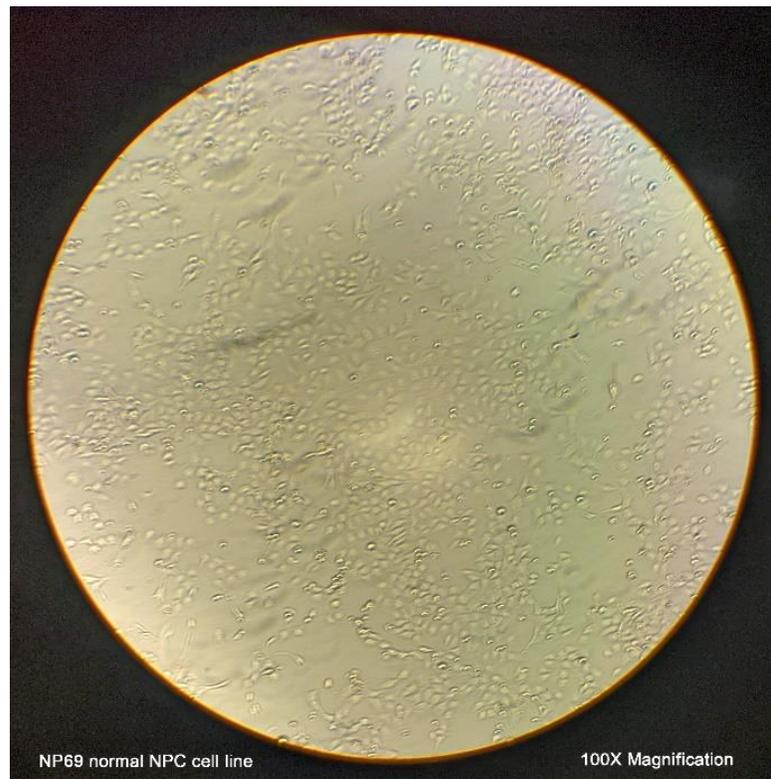


Figure 2: NP69 cell line viewed under microscope using 100x magnification.

2.2.2 SUNE-1

SUNE-1 cell line is known as a problematic cell line that derived from Homo sapiens (*Cellosaurus Cell Line*, 2015). It is widely used as *in vitro* model system in medical and biological research (Ye et al., 2015). Figure 3 shows the image taken in Animal Tissue Culture Laboratory of observing the SUNE-1 cell lines under 100x magnification using microscope.

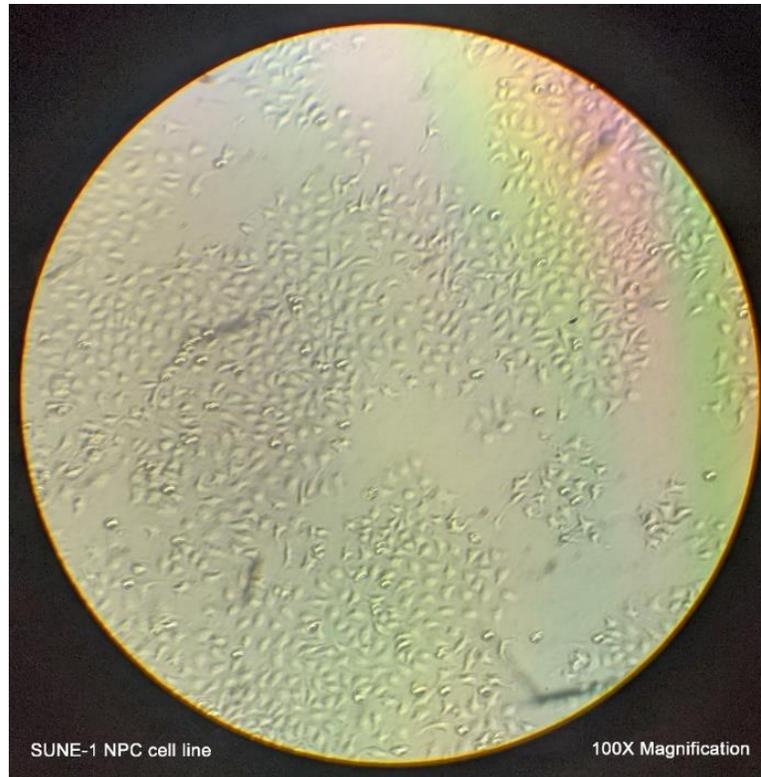


Figure 3: SUNE-1 NPC cell line observed under microscope using 100x magnification.

2.3 Tumor Suppressor Gene

2.3.1 p53 Family of Gene

p53 family gene comprised of TP53, TP63, and TP73 genes. Each of the genes construct protein isoforms across various mechanisms including substantial alternative of mRNA splicing (Wang et al., 2008). These isoforms are found to play a vital role in the regulation of numerous biological processes in normal cells (Wei et al., 2012). It is stated that the abnormal expression of these tumor protein genes contributes to tumorigenesis or carcinogenesis and has a profound effect on the response of tumor to the curative therapy. p53 is regulated in cell proliferation, differentiation, and apoptosis (Wei et al., 2012).

2.3.2 p73 Gene

P73 gene is a member of the p53 family gene, located on human chromosome 1p36 (Rufini et al., 2011), a region that constantly deleted in tumors for instance neuroblastoma and other advanced stage cancers, hence conclude that p73 is related to cancer (Kaghad et al., 1997). According to Joerger et al. (2009), p73 and p53 have different oligomerization domain structure. Due to this factor, p73 and p53 do not produce heterotetramers, which elaborates more about human delta Np73 inhibits p53 competitively for binding of promoter and not via direct protein to protein interaction (Rufini et al., 2011).

2.4 Housekeeping Genes

Housekeeping genes are known as cellular maintenance genes that required for the basic cellular function maintenance (Eisenberg & Levanon, 2003). It is expressed mostly in the cells of an organism under a normal condition. It is widely used in experimental purposes. The expression of housekeeping genes mainly used as a reference for the observation of expression analysis of other genes.

2.4.1 GAPDH

Glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*) play a crucial role in catalyzing the redox reaction in glycolysis by converting glyceraldehyde-3-phosphate into 1, 3-biphosphoglycerate via NAD^+ reduction to NADH (Tang et al., 2012). Hence, it serves as a transferrin receptor and glycolytic enzyme (Zhang et al., 2015). The expression status of *GAPDH* varies in different cell lines although it is commonly used as an internal control (Caradec et al., 2010).

2.5 Bibliographic Analysis

2.5.1 The Application and Significance of Bibliographic Analysis

Bibliographic analysis has earned a vast popularity in the business research field through the recent years (Khan et al., 2021). Bibliometric is defined as the application of statistical and mathematical methodology to the public publications (Rousseau, 2014). The application of bibliographic analysis demands of a connecting point that are consists of the authors, journals, individual publications, or subject categories (Nettle & Frankenhuis, 2019). This quantitative method has been widely used to examine the distinct perspective of the publications within a designated subject area (Ellegaard & Wallin, 2015). Various software such as RStudio is designed for the bibliographic analysis purpose.

RStudio allow the users to encompass mathematical functionality, statistical algorithms, and the capability of visualization fulfilling the purpose of bibliometric analysis (Derviş, 2019). The popularity of bibliographic analysis rises throughout the years among the researchers for its utility in the quantitative analysis.

The benefits of bibliographic including its ability to justify and document researcher's role or the research team of a research project. As articles contain metadata for instance categories, publication data, author, and publications time, bibliographic analysis is conducted to extract those data and perform an additional test to evaluate the user's proposed model of topic (Lim & Buntine, 2016). The procedures cost of bibliographic are inexpensive and requires little time to use.

CHAPTER 3: MATERIALS AND METHODS

3.1 Bibliographic Analysis of p53-gene Family in Nasopharyngeal Carcinoma

3.1.1 Research Topic and Boundary Definition

A bibliographic analysis was conducted by using the words “p53” and “Nasopharyngeal Carcinoma” as the main input of the databases. Bibliographic study was aimed to assimilate the universal trends of the publications on the gene that carries a role as controller of apoptosis (cell death) and division of cells. The software RStudio version 2.0 was utilized to access to the biblioshiny web-interface to analyze the data exported from Scopus database.

Table 1: Software and database used in bibliographic analysis

Software	Database
RStudio version 4.2	Scopus

3.1.2 Data Source Selection and Data Retrieval

The search terms for the related journal articles of Scopus database are shown in Table 2. The relevant articles from the last 22 years (2000-2022) were strictly follows the following database indexes for a systematic data collection. The data are then downloaded from the Scopus database in. bibTex format compatible to the biblioshiny web-interface.

Table 2: Search terms and number of articles (n) found in Scopus database.

Database	Query Input	No. of Articles (n)
Scopus	TITLE-ABS (p53) AND TITLE-ABS (nasopharyngeal) AND PUBYEAR > 1999 AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (LANGUAGE , "English"))	X

3.1.3 Data Refinement using PRISMA Checklist

Data validation was carried out for the screening of published articles using the PRISMA Checklist 2020. PRISMA Checklist consists of a 27-item checklist and a four-phase flow diagram that can be accessed on PRISMA organization official website (<http://www.prisma-statement.org/>). Data refinement via PRISMA Checklist useful in critical evaluation of the published systematic reviews found on the databases to filter out the unrelated articles, minimizing the biased bibliographic analysis outcome. A flow diagram of the selected journal articles on p53 in nasopharyngeal carcinoma is as below (Diagram 1).

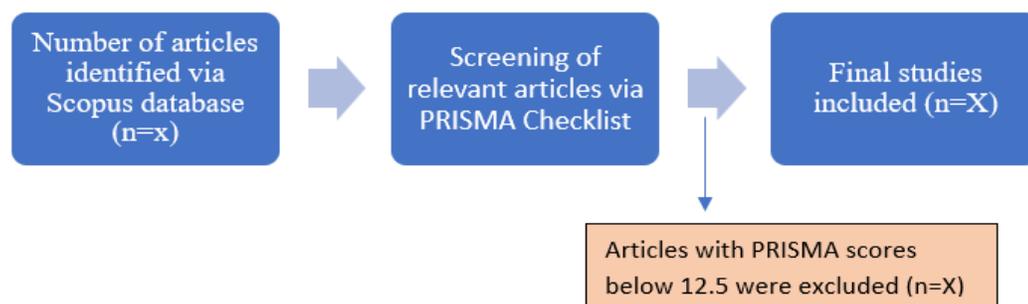


Diagram 1: A flow diagram of data extraction.

3.1.4 Analysis and Visualization of Findings

The final studies chosen were exported from each of the databases into a .bibtex format and imported into the biblioshiny web-interface. The plot or table of the annual scientific production, most relevant affiliations, corresponding author's country, and country scientific production were taken for the bibliographic visualization and analysis.

3.2 Cell Culture

3.2.1 Preparation of cell lines

NP69 NPC cell lines (Figure 4) and SUNE-1 (Figure 5) were utilized in this study. The SUNE-1 NPC cell line was cultured in the Roswell Park Memorial Institute (RPMI) growth medium alongside 1% penicillin antibiotics and 10% fetal bovine serum. The NP69 NPC cell line was cultured in a keratinocyte serum-free medium (KSFM), supplemented with bovine pituitary extract (BPE), human recombinant epidermal growth factor (EGF), and 1% penicillin antibiotics. The two cell lines both were incubated for growth in the same incubator settings, which were 37°C and humidified conditions with 5% carbon dioxide (CO₂). The cells were checked regularly to see if the confluency achieved between 70%-85% during their logarithmic growth phase.

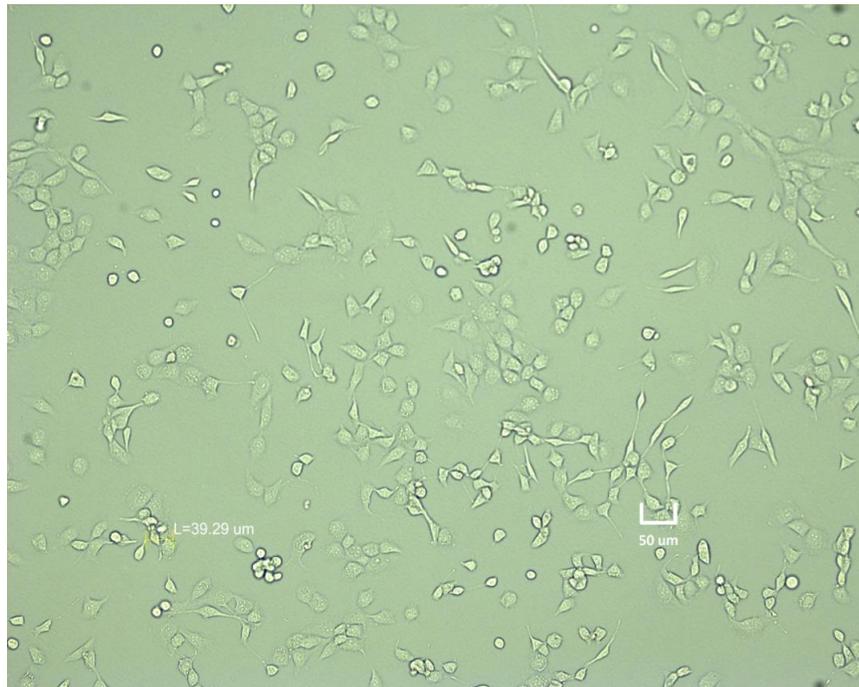


Figure 4: Microscopic Image of NP69 cell line.