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UNIVERSITI SAINS MALAYSIA

RUJUKAN

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R&D JANGKA PENDEK

UNIVERSITI SAINS MALAYSIA
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Bahagian R & D
Pusat Pengajian Sains Perubatan

"A PRELIMINARY STUDY OF p53 ONCOGENE EXPRESSION IN LUNG SAMPLES"

DR.MD.TAHMINUR RAHMAN
LECTURER, PATHOLOGY,
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...AGIAN PENYELIDIKAN
...PENGAJIAN SAINS PERUBATAN
...NAN :
Bhg. Penyelidikan, PPSP
Perpustakaan Perubatan, USMKK
RCMO
...an : ..... Tarikh : 18/12/03

Semua laporan kemajuan dan laporan akhir yang dikemukakan kepada Bahagian Penyelidikan dan Pembangunan perlu terlebih dahulu disampaikan untuk penelitian dan perakuan Jawatankuasa Penyelidikan di Pusat Pengajian.

USM R&D/JP-04

LAPORAN AKHIR PROJEK PENYELIDIKAN R&D JANGKA PENDEK

A. MAKLUMAT AM

Tajuk Projek: **"A preliminary study of p53 oncogene expression in lung samples."**

Tajuk Program:

Tarikh Mula: **01.08.2002**

Nama Penyelidik Utama: **Dr.Md.Tahminur Rahman** (M04084479)
(berserta No. K/P)

Nama Penyelidik Lain: **Dr.Hasnan Jaafar** (620501-08-6023)
(berserta No. K/P)

B. PENCAPAIAN PROJEK:

(Sila tandakan [/] pada kotak yang bersesuaian dan terangkan secara ringkas di dalam ruang di bawah ini. Sekiranya perlu, sila gunakan kertas yang berasingan)

This study was carried out to determine the level of expression of tumour suppressor gene p53 protein products among different types of lung lesions. The aim of this study was to find a scientific correlation between p53 expression indices and different type of lung lesions.

A total of 33 paraffin block lung samples of varying histological background were selected from Pathology department of three different hospitals of Malaysia. These cases comprised of both benign and malignant lesions of lung.

The result of the present study denotes the squamous malignancy as the most common phenotype for p53 positivity in lung samples and benign lesions are negative for p53 staining.

☐ **Rekaan atau perkembangan produk baru,**
(*Sila beri penjelasan makluman agar mudah dikomputerkan*)

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(2) _____

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☐ **Mengembangkan proses atau teknik baru,**
(*Sila beri penjelasan makluman agar mudah dikomputerkan*)

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☐ **Memperbaiki/meningkatkan produk/proses/teknik yang <u>sedia ada</u>**
(*Sila beri penjelasan makluman agar mudah dikomputerkan*)

(1) _____

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C. PEMINDAHAN TEKNOLOGI

☐ Berjaya memindahkan teknologi.

Nama Klien: (1) **Not applicable**
(Nyatakan nama penerima pemindahan teknologi ini dan sama ada daripada pihak swasta ataupun sektor awam)
(2) _____
(3) _____

☐ Berpotensi untuk pemindahan teknologi.
(Nyatakan jenis klien yang mungkin berminat)

This study will serve as a base line data for future work on p53 expression in lung samples

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D. **KOMERSIALISASI**

☐ Berjaya dikomersialkan.

Nama Klien: (1) **Nil**

(2) _____

(3) _____

☐ Berpotensi untuk dikomersialkan
(Nyatakan jenis klien yang mungkin berminat)

Nil

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E. PERKHIDMATAN PERUNDINGAN BERBANGKIT DARIPADA PROJEK

(*Klien dan jenis perundingan*)

(1) _____

(2) _____

(3) _____

(4) _____

F. PATEN/SIJIL INOVASI UTILITI

(*Nyatakan nombor dan tarikh pendaftaran paten. Sekiranya paten/sijil inovasi utiliti telah dipohon tetapi masih belum didaftarkan, sila berikan nombor dan tarikh fail paten*).

Presented as oral presentation in 8th Asia Pacific Association of Societies of Pathologists (APASP) congress held in Bali, Indonesia from 1-6 September 2003 and abstract published in the proceedings of the congress.

G. PENERBITAN HASIL DARIPADA PROJEK

(i) LAPORAN/KERTAS PERSIDANGAN ATAU SEMINAR

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(ii) PENERBITAN SAINTIFIK

(1) **A preliminary study of p53 oncogene expression in lung samples (submitted for publication in the Hongkong Medical Association Journal)**

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H. HUBUNGAN DENGAN PENYELIDIK LAIN

(sama ada dengan institusi tempatan ataupun di luar negara)

Local- Dr.Subathra Sabaratnam, Department of pathology,Hospital Pulau Pinang. Dr.Zakaria bin Jusoh,

(ii) PENERBITAN SAINTIFIK

(1) **A preliminary study of p53 oncogene expression in lung samples (submitted for publication in the Hongkong Medical Association Journal)**

(3) _____

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(6) _____

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H. HUBUNGAN DENGAN PENYELIDIK LAIN

(sama ada dengan institusi tempatan ataupun di luar negara)

Local- Dr.Subathra Sabaratnam, Department of pathology,Hospital Pulau Pinang. Dr.Zakaria bin Jusoh, Department of Pathology,HKT

(2) _____

Abroad- Presented as an oral paper in 8th APASP congress in Bali 2-6 September 2003. Many questions were asked about the topic and interactions were made with other pathologists and physicians attending that congress.

_____

(4) _____

_____

I. **SUMBANGAN KEWANGAN DARI PIHAK LUAR**
(Nyatakan nama agensi dan nilai atau peralatan yang telah diberi)

(1) ____**Nil**_____

(2) _____

(3) _____

J. **PELAJAR IJAZAH LANJUTAN** **Nil**
(Nyatakan jumlah yang telah dilatih di dalam bidang berkaitan dan sama ada diperingkat sarjana atau Ph.D).

	Nama Pelajar
Sarjana	_____
	_____
	_____
Ph.D	_____
	_____
	_____

K. **MAKLUMAT LAIN YANG BERKAITAN**

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_____

Professor Zabidi Azhar Mohd. Hussin
Chairman of Research & Ethics Committee
School of Medical Sciences
Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian,
KELANTAN, MALAYSIA.

07/09/2003
Tarikh

Dr. Haji Hasnan Jaafar
Ketua Jabatan
Jabatan Patologi
Pusat Pengajian Sains Perubatan
Kampus Kesihatan
Universiti Sains Malaysia
16150 Kubang Kerian,
Kelantan,

07/9/03
Tandatangan

TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN
PUSAT PENGAJIAN

A preliminary study of p53 oncogene expression in lung sample

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Department of Pathology, Hospital Kuala Terengganu***

A preliminary study of p53 oncogene expression in lung sample

Dr.Md.Tahminur Rahman*, Dr.Hasnan Jaafar*, Dr.Subathra Sabaratnam,Dr.Zakaria bin Jusoh*****

Department of Pathology, University Sains Malaysia*
Department of Pathology, Hospital Pulau Pinang**
Department of Pathology, Hospital Kuala Terengganu***

Abstract:

This study was carried out to determine the level of expression of tumour suppressor gene p53 protein products among different types of lung lesions. The aim of this study was to determine a scientific correlation between p53 expression indices and different type of lung lesions.

A total of 33 paraffin block lung samples of varying histological background were selected from Pathology department of three different hospitals of Malaysia. These cases comprised of benign lesions like malformations, emphysema, bronchiactasis, abscess, fungus and malignant lesions like different types of lung carcinomas.

All the samples were submitted for routine Haematoxylin & Eosin (H&E) stains and Immunohistochemical (IHC) staining for five mutant and wild type of p53 protein products namely DO1, BP, Phosphatase, DO7 and 1801 purchased from Novocastra company, UK. The indirect IHC techniques

were employed. The results were classified high and low level of staining using visual analogue scale suggested by Dowell and Hall. Also we counted 1000 cells randomly from the highest area of positive staining and counted the number of cells positive for the IHC staining.

The clinical symptoms found in the record sheet of these 33 patients were also analysed and correlated with the histological findings. It was found that 8(50%) of the positive samples had history of loss of appetite(LOA),Loss of weight (LOW) and cough. Haemoptysis was present in 6(37.5%), fever and other constitutional symptoms in 3 (18.75%) cases. 2 cases (12.5%) were smokers and 2(12.5%) cases were asymptomatic.

The p53 expression was highest in squamous cell carcinoma (43.75%), followed by adenocarcinomas(31.25%)and metastatic lesions in lung (25%). The finding of the present study is similar to other studies which showed that the p53 staining was more in squamous cell (epidermoid) carcinoma 16 of 27(59.22%) non small cell carcinoma 22 of 46(47.8%), small cell carcinoma 8 of 30(26.6%),adenocarcinomas 6 of 19(37.57%)[2] .

The result of the present study denotes the squamous malignancy as the most common phenotype for p53 positivity in lung samples and benign lesions are negative for p53 staining. However, as the

sample size of this study is low the results should be interpreted cautiously and it is advocated that study with large sample size is required to substantiate these findings

Introduction:

Oncogene and p53 association

Oncogenes are genes that cause cancer. P53 is the most commonly mutated genes in human cancers. 50% of all human cancer contain cells with point mutations or deletions in both alleles of p53 gene. Over expression of p53 oncogenes are found in majority of melanomas, HPV induced cervical cancers, breast& colon cancers, some cases of carcinoma of lung, bladder and thyroid [1].

The gene for p53 is located in chromosome 17p, a frequent site of allele loss in many tumours including breast, colon, brain, lung. The p53 over expression is expressed at a level detectable by IHC in carcinomas of various sites including breast, colon, bladder and lung . Expression has been shown to correlate with poor prognosis breast cancer [2,3,4,5]. In colonic tumours p53 was expressed in 47% of cancers, 9% in adenomas and no expression normal mucosa [6].

P53 is recognised as the cells policeman and in fact tumour suppressor gene.

Loss of genetic informations on chromosome 17p locus where p53 is found common in all neoplasia. The most frequent malignancies are malignant melanoma(88%), testicular tumour (87%), colorectal carcinoma (58%). Lower in the list were leukaemias (13%), thyroid tumours(5%).

p53 accumulation was observed in 76% of 212 human malignant lesions including breast, colon, stomach carcinomas, melanomas, carcinoma of testis, urinary bladder carcinoma, uterine carcinoma and soft tissue sarcomas [7].

P53 is a molecular phospho protein with a molecular weight of 53kDa. Wild type is present in wide variety of normal cells, but the protein has a very short half life and thus present in only minute amounts, generally below the detection level by IHC.Somatic mutation of p53 gene is very frequent event in the development of human neoplasia and because mutant p53 proteins often are much more stable than wild type p53 proteins the mutant p53 accumulates in high level [8].

The normal p53 protein is unstable and difficult to quantitate .wild type p53 acts as modulator which can turn cruicial genes either on or off. It also inhibits DNA replication ,in the regulation of apoptosis [9].Wild type p53 behaves as a tumour suppressor ,mutant p53 behaves as a dominant transformimg oncogene.The abnormal p53 protein or the mutant p53 is

more stable than the wild type. The degraded p53 protein is functional equivalence of a mutated p53.This result in abnormal protein accumulating to levels where it can be detected by IHC in contrast to normal low levels. IHC provides a rapid , economical method for p53 alterations and can be used on cytologic, paraffinised and frozen sections

DNA tumour viruses produces proteins that bind to and functionally inactivate p53 protein. Wild type p53 possesses tumour suppressor activity while mutant protein does not. Mutant protein can act as a dominant transforming element in DNA leading to development of neoplasia. Wild type p53 can induce cell cycle arrest, mutant type does not.

P53 is probably is a transcriptional regulator, wild type can bind DNA in a sequence specific manner, mutant p53 cannot. Abnormalities of p53 is common in neoplasia Mutation of p53 gene is associated with conformational changes and altered protein ability. P53 has a role in apoptosis[9,10,11,12], after geno toxic insults.

P53 and lung cancer association

Bronchial carcinogenesis is characterized by accumulated gene abnormalities which ultimately leads to malignant transformation of bronchial epithelial cells,followed by invasion and metastasis. One of the most common and consistant of these genetic lesion is inactivation of p53 tumour suppressor gene by mutation and deletion.The frequency of p53 alteration in lung cancer highest in these subtypes of bronchial cancers that are most consistently associated with smoking ,especially small cell lung carcinoma and squamous cell carcinoma. The frequency is lower in adencarcinoma in which association with smoking although present is not as strong[13].

In a study of forty nine specimen of non malignant and malignant tumour of lung tissue examined for wild type, and mutant type p53,bcl-2 and apoptosis. Wild type p53 expression peaked in peritumoral and metaplastic samples, mutant p53, bcl-2 and

apoptosis were first detected in metaplastic lesion and increased with progression to carcinoma[14].

P53 tumour suppressor gene play an important role in the carcinogenic process of lung carcinoma. The presence of multiple mutations in p53 gene may explain higher incidence of lung carcinoma in patients with idiopathic pulmonary fibrosis(IPF)[15].

Also p53 expression was observed significantly more frequently in bronchiolar dysplasias with pneumoconiosis 13 of 23, than patients without pneumoconiosis 9 of 23[16].

P53 and bcl-2 oncoproteins are detectable immuno electron microscopically in lung cancer cells[17].

Endobronchial carcinoma develops through a continuum of morphologically recognizable pre neoplastic changes. No marker has been identified that can predict biological behavior of these lesions. 39 endobronchial lesions from patients without overt lung cancer were analysed by IHC for abnormal expression of p53 protein i.e suprabasal p53 expression. Clear suprabasal immunostaining was found in 2(12%) of hyperplastic or squamous

metaplastic lesions,in 1(10%) of the mildly or moderately dysplastic lesion and in 9(75%) of the severely dysplastic or carcinoma in situ (CIS) lesion. Suprabasal p53 immunostaining was found significantly more frequent in severe dysplasia or CIS ($p<0.01$).It was concluded that suprabasal p53 staining is associated with bronchial cancer and might have additive value to predict biologic behavior of pre neoplastic endobronchial lesions in the population risk of bronchial cancer[18].

Reviewing all the mentioned literature search results and keeping in mind that little work was done and published on p53 expression in lung samples in Malaysia the present study was undertaken with the following **objectives:**

1. To form a baseline data for future work in the research of lung cancer in Malaysia particularly in relation to p53 expression.
2. To identify the common lesions of lung who are p53 positive and their clinico pathologic correlation.

Methods and Results:

A total of 33 paraffin block lung samples of varying histological background were collected from Pathology department of four different hospitals (hospital Kuala Terengganu, pulau Pinang, KotaBharu and USM) of Malaysia. These cases comprised of benign lesions like malformations, emphysema, bronchiactasis, abscess, fungus and malignant lesions.All the samples were submitted for routine Haematoxylin & Eosin (H&E) stains and Immunohistochemical (IHC) staining for five mutant and wild type of p53 protein products namely DO1, BP, Phosphatase, DO7 and 1801 purchased from Novocastra company, UK. The indirect IHC techniques were employed. The results were classified high and low level of staining using visual analogue scale suggested by Dowell and Hall[19]. Also we counted1000 cells randomly from the highest area of positive staining and counted the number of cells positive for the IHC staining.

The clinical symptoms found in the record sheet of these 33 patients were also analysed and correlated with the histological findings. It was found that 8(50%) of the histologically proven malignant samples had history of loss of appetite(LOA),Loss of weight (LOW) and cough. Haemoptysis was present

in 6(37.5%), fever and other constitutional symptoms in 3 (18.75%) cases. 2 cases (12.5%) were smokers and 2(12.5%) cases were asymptomatic. The results are shown in table I, 2,3,4.

Table-1
Showing Final distribution of cases.

Total no of Samples	Sex MaleFemale N(%)	Race Malay/Chinese N(%)	Mean age ± SE	Benign Lung Lesion	Malignant Lung Lesion
N=33	M22(66.66%), F11 (33.33%) Ratio 1.7:1	Malay22(66.66%) Chinese11(33.33%)	48.27±3.50	N=17 (51.52%)	N=16 (48.48%)

Table 2
Showing provisional/histological diagnosis and IHC staining results

Total no of samples	Provisional/clinical diagnosis Benign N(%)	Malignant N(%)	Histological diagnosis Benign N(%)	Malignant N(%)	IHC staining with p53 antibodies -ve N(%)	+ve N(%)	
N=33	20 (60.60%)	13 (39.39%)	17 (51.51%)	16 (48.48%)	28 (84.85%)	5 (15.15%)	

Table 3
Showing clinical features, IHC pattern in malignant cases

Total no of samples	Total +ve for malignancy	Primary/Secondary	Clinical features	IHC pattern +ve for p53 staining
N=33	N=16 (48.48%)	12 (75%) 4 (25%) SCC=7 Adeno Car=5	LOW } LOA }8(50%) Cough} Haemoptysis 6(37.5%) SOB5(31.25%) Fever3(18.75%) Smoker Asymptomatic 2(12.5%)	5(31.25%)

Table 4
Showing p53 IHC positivity pattern in 5 positive cases

Total no.of positive cases	% IHC +ve	anti bodies used (No.+ve cells/1000) DO1	DO7	BP	Phosphate	1801	Histological diagnosis
	<20%	56	123	110	52	48	SCC(WD)
N=5	<20%	415	238	185	177	240	Adeno Ca
	60%	652	365	672	--	----	PDSCC
	<20	--	410	350	--	----	PDSCC
	<20	66	57	28	--	----	Met GCT

Discussion

The present study showed p53 expression was highest in squamous cell carcinoma (43.75%), followed by adeno carcinomas(31.25%)and metastatic lesions in lung (25%).Of the five p53 antibodies used in the present study four were mutant forms namely DO1,DO7,BP,1801 and the fith one phosphate was a phosphorylated p53 antibody. The five positive cases show strong reactivity with DO7, BP; four out of five showed strong reactivity for DO1, two showed mild positivity for phosphate and 1801 antibodies. The highest positivity of staining was found in two poorly differentiated carcinomas, followed by squamous cell carcinoma, adenocarcinomas and mild positivity by metastatic giant cell tumour. This finding indicates that p53 expression is different in different type of lung cancer and there is mutation in different axons.

The finding of the present study is nearly similar to other study which using DO7 a p53 mutant type of antibodies showed that the p53 staining was more in squamous cell

(epidermoid) carcinoma 16 of 27(59.22%), non small cell carcinoma 22 of 46(47.8%), small cell carcinoma 8 of 30(26.6%), adenocarcinomas 6 of 19(37.57%)[20].

Other study showed over expression of p53 gene in pulmonary adenocarcinomas specifically associated with cigarettes smoking.[21] Over expression of p53 tumour suppressor gene product in pulmonary adenocarcinomas has been found to be specifically associated with cigarrette smoking and represent a very early event in the genesis of the tumour. Mutations results in the overexpression and thus detectibility of the protein by IHC.p53 appears to have both a function as an oncoprotein perhaps by complexing in its mutant form with wild type p53 and as a tumour suppressor gene in its normal state[22].

Although two cases were smoker in our study one was histologically diagnosed as well differentiated adenocarcinomas and the other poorly differentiated carcinoma. The poorly

differentiated carcinoma showed positive p53 staining while the well differentiated adenocarcinoma was negative. This is in partial concordance with the above mentioned studies.

Clinico pathological studies of p53 abnormalities in present study showed 50% (8 of 16) of the malignant cases presented with loss of appetite(LOA), loss of weight(LOW), 37.5%(6of 16) presented with haemoptysis, fever and other constitutional symptoms in 3 (18.75%) cases. 2 cases (12.5%) were smokers and 2(12.5%) cases were asymptomatic. Other studies showed conflicting results no correlation between p53 status and clinical presentations.

P53 expression may play a role as a prognostic marker in lung cancer particularly non small cell lung cancer(NSCLC). In a study of 179 patients with NSCLC when correlated with p53 staining using DO7 antibody showed patients with a strong expression of p53 oncoprotein(>50%) have favourable prognostic factor leading to a prolong survival[24]. The median survival time for strong and weak p53 expression was more than 61/44 months respectively[23].

Literature review stated that in prostate carcinoma p53 immuno reactivity has been reported to be associated with higher Gleason score, nuclear grade, pathologic stage and metastasis.[25]

In head neck squamous carcinomas p53 immuno reactivity has been correlated with increased risk of local regional recurrences, resistance to radiotherapy and risk of developing a second primary tumour.[26]In colorectal cancers it acts as an independent indicator of poor prognosis in some studies while in breast carcinoma lack of functional p53 is associated with aggressive biological factors and poor clinical out come.[27] In melanomas p53 expression is associated with an increase in the depth of invasion in the primary tumours, presence of metastasis[28]. In thyroid malignancies p53 reactivity is usually detected in anaplastic or poorly differentiated tumours.Normal thyroid does not express p53 mutations,while p53 is present in low level in follicular adenoma(14%),nodular hyperplasia(16.7%),papillary carcinoma (17%) indicates that p53 does not have a major role in the pathogenesis of papillary

carcinoma.[29]

But as our study was a retrospective one and because of the smaller sample size we could not study these parameters.

P53 is a multifactorial molecule and regulates cell cycle, apoptosis, angiogenesis. In a study of 32 NSCLC it was found that expression of p53 and VEGF was higher in mutant p53 +ve tumours than those express normal p53 that showed no p53 staining suggesting that the functional inactivation of p53 may up regulate VEGF expression.[30,31,32]

It has been showed that introduction of wild type p53 into mutant p53 gene via recombinant technique had significantly suppressed the VEGF protein expression. Also the expression of wild type p53 can induce inhibition of growth of tumours formed in nude mice due to induction of apoptosis in tumour cells. Direct injection of tumour in terminal lung cancer patients with such expressing wild type p53 gave same result and in some cases leads to regression. These results suggest that p53 gene replacement may be applicable for the treatment of human cancer as an pro apototic and

anti angiogenic therapy[33] As we only concentrated on p53 staining we could not correlate these findings.

Conclusion:

p53 is an important marker and p53 aberrations can have a role in diagnosis of neoplasia and could be employed to predict prognosis, recurrence and aggressiveness of the tumour in different sites like breast ,colon, melanoma, head and neck cancers, bladder and thyroid cancer etc.

As literature search found little work done on lung samples in Malysia to detect expression of p53 oncogene, we thought it would be useful if we could do a base line study. We collected already existing lung samples in different histopathological laboratories in four teaching hospitals in Malaysia. We could only manage to get 33 lung samples covering different benign and malignant lesions and stained them with p53 antibodies and examined it under light microscope.

We found that majority of the benign lung lesions does not

express p53, while a significant portion of malignant lesions over express p53 mutant proteins. Although these findings are not statistically significant because of the low sample size still it can be used as a base line data for research in Malaysia.

Limitations:

Inspite of our sincere effort to collect at least 100 lung samples we were able to get 60 lung samples from four different hospitals in Malaysia. Finally we could do p53 staining on only 33 samples, the rest 27 samples were not suitable for our study because of non availability of tissues after trimming. Also as this was a retrospective study using already available paraffin blocks of lung samples many areas of investigation like prognostic, behaviorial aspect of lung tumour were not possible to ascertain.

A further study, both prospective and retrospective with larger number of lung samples is advocated to find out the importance of p53 expression in lung carcinomas in terms of prognosis, behavior, recurrence, metastasis which will ultimately help better management, early diagnosis and influence the survival of lung

cancer patients.

Also we advocate that IHC staining of biopsy samples using different antibodies should be used routinely alongside of H&E stain for better diagnosis of malignant lesions.

Acknowledgement

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