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rasP-21, C-erbB-2 AND P-53 IMMUNOREACTIVITY AS A MALIGNANCY PREDICTOR OF THE PLEOMORPHIC PAROTID GLAND ADENOMA'S

Achmad Syawqie Yazid

Senior Consultant.Anatomical Pathology Department
Faculty of Dentistry and Faculty of Medicine
Padjadjaran University

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Abstract

Pleomorphic parotid gland adenomas (PPA's) have a variability of histopathologic appearances, making it difficult to classify and its biological behavior is also difficult to predict. To find a better understanding of this phenomenon a retrospective causal study was undertaken on 25 benign and 17 malignant PPA's cases. Having exposed the underlying process it is hoped that it can be applied for the prediction of the malignant changes occur in PPA's depending on the expressions of oncogenes rasP-21, C-erbB-2 and P-53 immunohistochemically. The immunoexpression were defined by the criteria: 0 = negative; +1=focal (<20%); +2=heterogenous (20-50%); and +3=diffuse (>50%). Heterogenous and diffuse are considered to be an overexpression criterias. The immunoexpression percentage of ras P-21 (88.1%), C-erbB-2 (92.8%) and P-53 (97.6%) is highly significant ($p<0.01$) in emerging the variability of the biological behaviour of PPA's. The sensitivity of immunohistochemistry and $Pv^{negative}$ (both 100%) are indicative of their abilities for detecting the malignant potentials of PPA's. The spesifisity of immunohistochemistry of 50%, and the presence of false positive in the benign PPA's cases could be considered of having the potentials for malignant change. Apparently overexpression of the rasP-21, C-erbB-2 and P-53 plays a role as a dependable indicator of having the potentials of benign PPA's for malignant change.

Key words: Pleomorphic parotid gland adenomas, rasP-21, C-erb B-2, P-53

Introduction

Pleomorphic adenoma is the most common benign tumor of the salivary gland[1], and most frequent in the parotid gland of women in the fourth decade of life, and young and older men.[2] Although pleomorphic parotid gland adenomas (PPA's) tends to be well circumscribed, small extensions can be seen protruding into the adjacent normal tissue.[1,2] Since the term PPA's tumor first introduced by Billroth in 1859 as a tumor consisting of four different kinds of tissue[3], make pathologists and clinicians until now are

often confused by differs on their biological behavior. Aside from being often recurrent after surgery, PPA's can transform into malignant, and sometimes can metastasizing al-though histological appears it is still benign.[4] With respect to the varying clinical manifestation, it is often difficult to predict their biological behavior. The morphology atypia[5] such as enlarged, hyperchromatic nuclei and frequent or abnormal mitosis can not be used as a parameter to predicting the malignancy potential.

To propose the predictor of malignancy potential by estimating the rasP-21, C-erbB-2, and P-53 oncogenes expression, which is said as being related to tumorigenesis, agressiveness and prognosis [6,7,10,12,13], by immunohistochemical method[14] that is sensitive, specific and rapid to perform together with the routinely paraffin embedded tissue examination making it more clinically available.

Materials And Methods

Fourty two cases of PPA's collected from 1980 through 1996 were obtained from the files at the Department of Anatomic Pathology of the Faculty of Medicine Padjadjaran University/Hasan Sadikin Central General Hospital; Kebonjati Hospital; Advent Hospital; and St.Boromeus Hospital Bandung; Sebelas Maret University Solo, and from The Department of Maxillofacial Surgery Kobe University School of Medicine Japan. All the material of study is included in sampling from time continuum, and restudied for histopathologic diagnoses and clinicopathologic recording, that is: tumor size, local infiltration, and regional lymph node status.

Immunohistochemistry was performed on deparaffinized, 5μm sections after antigen retrieval using microwave oven heating and $AlCl_3$ buffer solution. The anti-body used are a mouse monoclonal antibody rasP-21 (Dako Corp.CA.USA); c-erbB-2 and DO7 antihuman p53 protein (Novocastra Lab.Ltd.,UK). The immunohistochemical staining is performed with Novostain Super ABC kit (Novocastra Lab.Ltd.UK).

In brief, after deparaffinisation, antigen retrieval and inactivation of endogenous per-oxidase activity and blocking of cross reactivity the sections were incubated for: during 1 hour for rasP-21 and P-53 at room temperature, and for 24 hours for c-erbB-2 at 4°C. Localization of the primary antibody was achieved by subsequent incubation of biotinylated antiprimary antibody with an avidin-biotin-complex conjugated horse-radish peroxidase and diaminobenzidine.

Negative tissue control is the normal salivary gland beyond the tumor mass (internal tissue control). Positive rasP-21 control is lymphocyte cells in PPA's tumor; positive C-erbB-2 staining control uses breast tissue of Paget's disease; while P-53 staining control uses breast ductal carcinoma. The immunostaining pattern was assessed - who were unaware of patients clinical status - on the basis of extent that is percent of positive tumor cells, as : **0** = negative, no staining.; **1**$^{+}$ = focal staining pattern, present in <20% of tumor cells; **2**$^{+}$ = hetrogenous staining, present in 20-50% of tumor cells; **3**$^{+}$ = diffuse staining, present in >50% of tumor cells. Heterogenous and diffuse immunostaining are the criteria for overexpression of rasP-21, C-erbB-2, and P-53.

Results

From 42 PPA's cases collected on the whole during the period 1980-1996 (sampling from time continuum) 25 among others are PPA's tumors diagnosed as benign histopathologically, and 17 cases are malignant PPA's tumors histopathologically and clinically. Sixteen malignant cases and 21 benign cases are surgery treated, while 1 malignant case and 4 benign cases are biopsy treated (Table 1). The age span of subject is between 12 to 66 years with details 18 male cases with an

age span from 12–63 years. 24 female cases with an age span from 19-66 years.

The immunoexpression percentage of rasP-21 is 88.1%; C-erbB-2 is 92.8%; and P-53 is 97.6% tested on a probability of H_0: $\pi = 0.5$ and H_1 : $\pi > 0,5$ by using Ztesting. Z-count>one way Z-table at a significancy of $p<0.01$, it is empirically evident that the immunoexpression of rasP-21, C-erbB-2, and P-53 can cause emerging the variability of the PPA's tumor biological behavior (Table 2).

The sensitivity of ABC-IHC immunoexpression of rasP-21, C-erbB-2, and P-53 test has achieved 100%, indicating the high competence of the test for detecting the malignancy potential of the PPA's tumor (Table 3).

The specificity of IHC-ABC test rasP-21 of 68%, C-erbB-2 of 48%, and P-53 of 56% indicate that the three oncogene-proteins expression can be detected either in benign or malignant PPA's cases but with a differ expression composition (table 3). Examination result of false-positive observed in benign PPA's cases reminds us that benign PPA's have a large potential to transform into malignant (Table 3). While the risk opportunity of PPA's became malignant calculated through logistic regression analysis, indicate that the risk opportunity of the malignancy would increase along with the increase of those three oncogene-proteins immunoexpression (Table 4 and 5).

Discussion

Comprehensive study of rasP-21, C-erbB-2, and P-53 immunoexpression of the benign PPA's tumor and malignant PPA's tumor as a gold standard that has been done has succeeded in propose the potential prediction parameter of tumor malignancy in answering the specifying basics of the apoptosis impairment as a critical component[15] of PPA's

Table 1. Clinical and histologic feature, and rasP-21, C-erbB-2, P-53 immunoexpression of the pleomorphic parotid gland adenoma's.

No	Slide registeration number	Sex / Age	Clinical diagnosis / (Biopsy / Operation)	Histological Diagnosis	Immunoexpression		
					rasP-21	C-erbB-2	P-53
1	96174 / RSK	F/35	Parotid tumor / O	Malignant pleomorphic adenoma	3 (H)	3 (H)	3 (H)
2	901983 / RSHS	F/60	Benign parotid tumor / B	Malignant pleomorphic adenoma	3 (H)	3 (H)	3 (H)
3	92572 / RSK	M/27	Parotid tumor / O	Malignant pleomorphic adenoma	3 (G)	3 (G)	3 (G)
4	951819 / RSHS	F/63	Malignant parotid tumor/O	Malignant pleomorphic adenoma	2 (H)	2 (H)	3 (H)
5	862490 / RSHS	M/46	Salivary gland tumor/ O	Malignant pleomorphic adenoma	2 (G)	3 (G)	3 (H)
6	960877 / RSHS	M/52	Malignant parotid tumor/O	Malignant pleomorphic adenoma	3 (G)	3 (H)	3 (H)
7	881023 / RSHS	M/24	Recurent parotid tumor / rs	Malignant pleomorphic adenoma	3 (G)	3 (G)	3 (G)
8	966079 / UNS	F/52	Recurent parotid tumor / rs	Malignant pleomorphic adenoma	2 (G)	2 (G)	2 (G)
9	966816 / UNS	M/44	Recurent parotid tumor / rs	Malignant pleomorphic adenoma	3 (G)	3 (G)	3 (G)
10	Oey-1 / TYM	M/59	Parotid tumor / O	Malignant pleomorphic adenoma	3 (G)	3 (G)	3 (G)
11	Oey-2 / TYM	F/43	Parotid tumor / O	Malignant pleomorphic adenoma	3 (G)	2 (G)	3 (G)
12	Oey-3 / TYM	F/47	Parotid tumor / O	Malignant pleomorphic adenoma	2 (G)	3 (G)	3 (H)
13	91992 / KOBE	F/45	Parotid tumor / O	Malignant pleomorphic adenoma	2 (G)	3 (H)	2 (G)
14	95494 / KOBE	M/63	Parotid tumor / O	Malignant pleomorphic adenoma	3 (G)	3 (H)	3 (G)
15	96677 / KOBE	F/53	Parotid tumor / O	Malignant pleomorphic adenoma	3 (H)	3 (H)	3 (H)
16	961986 / KOBE	F/66	Parotid tumor / O	Malignant pleomorphic adenoma	3 (H)	3 (H)	3 (G)
17	962061 / KOBE	F/62	Parotid tumor / O	Malignant pleomorphic adenoma	2 (G)	3 (H)	3 (G)
18	953060 / RSHS	M/27	Parotid tumor / O	Benign pleomorphic adenoma	1 (G)	2 (H)	2 (G)
19	94352 / RSK	M/40	Parotid tumor / O	Benign pleomorphic adenoma	2 (H)	2 (G)	3 (G)
20	931134 / RSK	M/45	Parotid tumor / O	Benign pleomorphic adenoma	2 (H)	2 (G)	1 (G)
21	95694 / RSK	F/20	Parotid tumor / O	Benign pleomorphic adenoma	1 (G)	2 (H)	2 (H)
22	831941 / RSHS	M/34	Parotid tumor / O	Benign pleomorphic adenoma	1 (H)	1 (G)	1 (G)
23	952112 / RSHS	F/57	Parotid tumor / O	Benign pleomorphic adenoma	1 (G)	2 (H)	2 (G)
24	91006 / RSK	M/40	Parotid tumor / O	Benign pleomorphic adenoma	1 (G)	1 (G)	1 (G)
25	92926 / RSK	F/55	Parotid tumor / O	Benign pleomorphic adenoma	3 (G)	3 (G)	3 (G)
26	851850 / RSHS	M/18	Parotid tumor / O	Benign pleomorphic adenoma	0	1 (G)	1 (G)
27	854533 / RSHS	M/18	Parotid tumor / O	Benign pleomorphic adenoma	1 (G)	2 (H)	2 (G)
28	960543 / RSB	F/19	Parotid tumor / B	Benign pleomorphic adenoma	2 (H)	2 (H)	2 (H)
29	854751 / RSHS	F/65	Parotid tumor / O	Benign pleomorphic adenoma	1 (G)	1 (G)	2 (G)
30	91694 / RSK	M/54	Parotid tumor / O	Benign pleomorphic adenoma	0	2 (H)	2 (G)
31	847531 / RSHS	F/25	Parotid tumor / O	Benign pleomorphic adenoma	1 (G)	1 (H)	1 (G)
32	941216 / RSK	F/47	Parotid tumor / O	Benign pleomorphic adenoma	2 (G)	2 (H)	1 (H)
33	814292 / RSHS	M/24	Parotid tumor / O	Benign pleomorphic adenoma	2 (G)	2 (H)	3 (G)
34	830396 / RSHS	F/31	Parotid tumor / O	Benign pleomorphic adenoma	1 (G)	1 (H)	1 (H)
35	813167 / RSHS	F/55	Parotid tumor / B	Benign pleomorphic adenoma	0	0	0
36	951123 / RSB	F/51	Parotid tumor / O	Benign pleomorphic adenoma	0	2 (H)	1 (G)
37	891060 / RSHS	M/35	Parotid tumor / O	Benign pleomorphic adenoma	1 (H)	1 (H)	1 (G)
38	831735 / RSHS	F/25	Parotid tumor / B	Benign pleomorphic adenoma	2 (H)	1 (G)	1 (G)
39	812502 / RSHS	F/40	Parotid tumor / O	Benign pleomorphic adenoma	1 (G)	0	1 (G)
40	952941 / RSHS	F/45	Parotid tumor / O	Benign pleomorphic adenoma	1 (G)	0	1 (G)
41	831134 / RSHS	M/12	Malignant parotid tumor/B	Benign pleomorphic adenoma	2 (H)	1 (G)	2 (G)
42	836858 / RSHS	F/45	Parotid tumor / O	Benign pleomorphic adenoma	0	1 (G)	1 (H)

Note :
F = female; M = male; H = homogenous; G = granuler RSHS = hasan sadikin central general hospital Bandung-Indonesia
RSK = kebonjati hospital; RSB = St Boromeus hospital; RSA = advent hospital Bandung-Indonesia;
KOBE = Kobe teaching hospital Japan; UNS = sebelas maret university Solo-Indonesia.
TYM = Dep.of Pathology kebonjati hospital Bandung-Indonesia; O = surgery; rs = radical surgery; B = biopsy

Immunoexpressions	Percentages	Tested with Ho: = 0.5 H1: > 0.5		
		Z-count	Z-table	DoS
1. rasP-21	88.1	4,948	2.33	p<0.01
2. C-erbB-2	92.8	5,558	2.33	p<0.01
3. P-53	97.6	6,182	2.33	p<0.01

Note : DoS=degree of significancy.

Table 2. The percentages of the ras P-21, C-erbB-2, and P-53 on the malignant and benign pleomorphic parotid gland adenomas (N=42)

Immunoexpression examination		Diagnoses		Calculation
		Malignant	Benign	
rasP-21	Overexpression (diffuse and heterogenous)	17 "true +"	8 "false +"	Sensitivity = 17/17 = 100% Spesificity = 17/25 = 68%
	Not Overexpression (Negative and Focal)	0 "false -"	17 "true -"	Accuracy = 34/42 = 80.9% PV+ = 17/25 = 68% PV- = 17/17 = 100%
C-erbB-2	Overexpression (diffuse and heterogenous)	17 "true +"	12 "false +"	Sensitivity = 17/17 = 100% Spesificity = 12/25 =48%
	Not Overexpression (Negative and Focal)	0 "false -"	13 "true -"	Accuracy = 30/42 = 71.4% PV+ = 17/29 = 58,6% PV- = 13/13 = 100%
P-53	Overexpression (diffuse and heterogenous)	17 "true +"	11 "false +"	Sensitivity = 17/17 = 100% Spesificity = 14/25 = 56%
	Not Overexpression (Negative and Focal)	0 "false -"	14 "true -"	Accuracy = 31/42 = 73.8% PV+ = 17/28 = 60.7% PV- = 14/14 = 100%

Note
Diagnoses = histopathology diagnoses with clinically confirmations

Table 3. Correlation between the expression of the ras P-21, C-erbB-2 and P-53 with pleomorphic parotid adenomas histopathology and clinically diagnoses

Variable	B	S.E.	Wald	df	Sig.	R	Exp(B)
rasP-21	1. 3201	1. 2303	1. 1514	1	0.2832	0.0000	3. 7439
C-erbB-2	2. 4306	1. 3603	3. 1930	1	0.0740	0.1451	11. 3660
P-53	0.8995	1. 1022	0.6660	1	0.4144	0.0000	2. 4583
Constanta	-105 774	3. 3449	9. 9959	1	0.0016		

Table 4. Multiple logistic regression analysis :ln{Y/(1-Y)} = -10.5754 + 1.3201 rasP-21 + 2.4306 C-erbB-2 + 0.8995 P-53 (accuracy = 92.8%).

tumorigenesis, malignancy development, and biological behavior variability.

A rasP-21 positivity percentage which is far more smaller than the C-erbB-2 and P-53 strongly suggest that rasP-21 has a role in the initial stage of the tumorigenesis and malignant transformation, while the P-53 and C-erbB-2 are involved in the further stage of the malignancy. But by considering the direct action of P-53 as an regulator of the apoptosis impairment process[15,16] suggest that P-53 also plays a big part in the initial stage along with rasP-21.

Some findings of immunoexpression rasP-21, C-erbB-2, and P-53 and staining characteristics in the studied PPA's supporting the above assumption among others are :

(a) Forty-eight percent of benign PPA'S has a focal rasP-21 immunoexpression with granulary dominant staining, and 52% benign PPA's has a focal P-53 immuno-expression with granulary dominant staining, indicate that rasP-21, and P-53 involved in the initial stage of the PPA's tumorigenesis.

(b) Forty-four percent of benign PPA's has a heterogenous C-erbB-2 immunoexpression with homogenous dominant staining, which indicate that C-erbB-2 is involved in the advanced

P-53	C-erbB-2	rasP-21	Probability of malignancy / p(Y)
0	0	0	0,000025
0	1	1	0,001085
0	1	2	0,004051
0	1	3	0,015001
0	2	1	0,012199
0	2	2	0,044192
0	2	3	0,147555
0	3	2	0,344478
0	3	3	**0,663001***
1	0	0	0,000062
1	1	1	0,002664
1	1	2	0,00901
1	1	3	0,036089
1	2	1	0,029466
1	2	2	0,102063
1	2	3	0,29851
1	3	2	0,563677*
1	3	3	**0,828665***
2	0	0	0,000154
2	1	1	0,006524
2	1	2	0,023995
2	1	3	0,084285
2	2	1	0,069454
2	2	2	0,218402
2	2	3	**0,511273***
2	3	2	**0,760532***
2	3	3	**0,922420***
3	0	0	0,000379
3	1	1	0,015887
3	1	2	0,056995
3	1	3	0,184522
3	2	1	0,15504
3	2	2	0,407212
3	2	3	**0,720027***
3	3	2	**0,886461***
3	3	3	**0,966920***

Note : Number with asterix shows the probability of malignancy greater than 0.5 that is having big malignancy potentials.

Table 5. Probability of malignancy :
$p(Y) = 1 / \{1 + \exp-(\alpha+\beta_1X_1+\beta_2X_2+\beta_3X_3)\}$.

stage of PPA's tumorigenesis and malignancy development.

(c) Eighty-eight percent of malignant PPA's has a diffused P53 immunoexpression and 82% has a diffused C-erbB-2 immunoexpression, both are homogenous, indicating that together both are involved in the advanced stage of the PPA's malignancy and tumorigenesis development.

(d) The staining pattern of C-erbB-2 75% localized in membrane-cytoplasm-apical, and P-53 localized in nucleus-cytoplasm, is observed in PPA's tumor that having a high proliferation activity.

The observation of several clinicopathological parameter that related and enforcing the above assumption is :

a) Benign PPA's having an overexpression of rasP-21, C-erbB-2, and P-53 appears to be progressive, has a big growth potential (tumor sizes > 4 cm).

b) Malignant PPA's with overexpression of rasP-21, C-erbB-2, and P-53 tends to be clinically aggressive, indicated by expansive infiltrative growth and metastasize to the regional lymph node.

The possible mechanism of action of rasP-21, CerbB-2, and P-53 in PPA's tumorigenesis, malignancy, and biological behavior variability that has been studied is lies in its potentials to interfere with signals creating confusion in the cell cycle and apoptosis. The result is that the apoptosis process as a neoplastic growth inhibitory doesn't progress and promotes the tumorigenesis, malignancy development, and PPA's biological behavior variability. The initial stage of PPA's tumorigenesis is promoted by rasP-21 and P-53; while advanced tumorigenesis, malignancy and

biological behavior variability is promoted by P-53 and C-erbB-2.

The biological behavior variability of PPA's is observed in 3 malignant PPA's cases ex recurrent benign PPA's tumor, and in 2 cases when diagnosed indicate expansive growth into the adjacent normal tissue surrounding salivary gland, and metastasize to the regional neck lymph node. These findings enforce the preassumption that the overexpression of rasP-21, C-erbB-2, and P-53 causes the variability of the PPA's biological tumor behavior, based on metastasizing to the lymph node as a prognosis potential parameter, infiltration to surrounding tissues and a tumor size of more than 4 cm as an aggressiveness potential parameter, which entirely can be considered as an advanced tumorigenesis parameter of PPA's.

All benign PPA's cases observed in this study can not indicate even one with signs of malignancy. In hypercellular PPA's cases there is no abnormal cytomorphologic features is observed, such as high mitotic rate. The availability of neoplastic tissue within the fibrous capsule (one of the tumor malignancy criteria) is observed in 1 case, but its tumor mass does not indicate the abnormal cytomorphologic atypia. So that in the absence of abnormal cytomorphologic atypia the hypereellularity and capsular invasion is an acceptable features in benign PPA's tumor. Considering the expression of rasP-21, C-erbB-2, and P-53 on the benign PPA's observed above have negative immunoexpression grade until diffused which is known as related to tumorigenesis and tumor aggressivity, so that it is evident that the histopathologic morphology criteria can not be used for predicting the malignancy potential of PPA'S tumor.

Conclusion

The overexpression of the oncogene rasP-21, C-erbB-2 and P-53 can impair the apop-tosis process, leading to the borderline malignancy development of the benign PPA's.

The immunoreactivity of rasP-21, C-erbB-2, and P-53 could be usefully used as a tool of prediction parameter for the malignancy potential of benign PPA's tumor that is often difficult to predict based on clinically and histopathology morphology criteria

References

1. Ellis GL and Auclair PL. *Atlas of tumor pathology*. 3rd series. Fasicles 17: Tumors of the salivary glands. Armed forces Institute Of Pathology. Washington D.C. 1996. pp.1-57,228-51.
2. Rosai J. *Ackerman's Surgical Pathology*. Vol.1. 7thed. The CV Mosby Co. St. Louis,Toronto,Washington DC. 1989. pp.642-7.
3. Sunardhi-Widyaputra.S. *Immunohistochemical studies on pleomorphic adenoma of the salivary gland*. Thesis. 1995. Katholieke Universiteit Leuven. Belgium.
4. Wenig BM, Hitchcock CL, Gnepp DR. Metastasizing mixed tumor of salivary glands: a clinicopathologic and flow cytometric analysis. *Am.J.Surg.Pathol.* 1992.16(9): 845-58.
5. Seifert G. Histopathology of malignant salivary gland tumours. *Oral Oncology Eur.J.Cancer*. 1992. 28 *B* (1): 49-56.
6. Gallo O, Franchi A, Bianchi S, Boddi V, Giannelli E, Alajmo E. p53 onco-protein expression in parotid gland carcinoma is associated with clinical outcome. *Cancer* 1995. 75: 2037-44.
7. Rosa JC, Fonseca I, Felix A, Soares J. p53 immunoexpression in carcinomas arising in pleomorphic adenoma. *Int.J.Surg.Pathol.* 1996. 3(4): 257-62.
8. Righi P.D., Li YQ, Deutsch M, et.al. The role of the p-53 gene in the malignant transformation of pleomorphic adenoma of the parotid gland. *Anticancer-Res.* 1994. 14 (5B): 2235 - 7.
9. Okutsu S, Takeda A, Suzuki T, et.al. Expression of rasP-21 and *ras* gene alteration in pleomorphic adenomas. *J.Nihon univ.Sch.Dent* 1993. 35: 200-3.
10. Harnowo S. *Flow cytometri DNA histogram profile and ras gene expression as an malignancy indicator of pleomorphic adenoma of the parotid salivary gland.*

Disertation. 1995. Gajah Mada University.

11. Milasin J, Pujic N, Dedovic N, et.al. H-ras gene mutations in salivary gland pleomorphic adenomas. *Int.J.OralMaxillofac.* 1993. 22: 359 - 61.
12. Sugano S, Mukai K, Tsuda H, et al. Immunohistochemical study of c-erbB-2 oncoprotein expression in human major salivary gland carcinoma: an indicator of aggresiveness. *Laryngoscope.* Aug. 1992. 102: 923-7.
13. Muller S, Vigneswaran N, Gansler T, Gramlich T, deRose PB., Cohen C. c-erbB-2 oncoprotein expression and amplification in pelomorphic adenoma and carcinoma ex-pleomorphic adenoma: a relationship to prognosis. *Modern Pathology.* 1994. 7(6): 628-31.
14. Leong AS-Y, Robbins P, Spagnolo DV. *Relevance and detection system of tumor genes and their proteins in cytologic and surgical specimens.* 1995. Jakarta international cancer conference.
15. Kastan MB, Canman CE, Leonard CJ. P53, cell cycle control and apoptosis: implication for cancer. *Cancer and Metastatic Review.* 1995. 14: 3-15.
16. Cummings MC, Winterford CM, Walker NI. Apoptosis. *Am.J.Surg. Pathol.*1997. 2 (1): 88 -101.