International Journal of Applied Engineering Research ISSN 0973-4562 Volume 13, Number 10 (2018) pp. 7874-7881

The Correlation of P53 Expression and Trombospondin-1, and the Expression Difference of *Trombospondin-1* Stadium III and IV on the Nasopharyngeal Carcinoma WHO Type 3

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Abstract

This study is as analitic observational research by cross sectional design. There are 24 samples from the tissue biopsy of Nasopharyngeal Carcinoma (NPC) WHO type 3. The purpose of this reseach to investigate correlation of P53 and *Trombospondin-1* expession stadium III and IV on the NPC WHO tipe 3. Each of them are carried out the observation of P53 and *Trombospondin-1* by using the examination of imunochemistry and then score of histology is evaluated by using Olysia software. The results show that the expression of P53 from 24 samples of NPC WHO type 3 has the average of 7.66 with the standard deviation of 0.95. however, the expression of *Trombospondin-1* has the average of 7.78 with the standard deviation of 1.21. Result of correlation test by Perason's product moment shows that the correlation coefficient (γ) is 0.473 with p value is 0.020 ($p < 0.05$). Different test of *Trombospondin-1* between stadium III and IV is 0.045 ($p < 0.05$).

Keywords: P53, *Trombospondin-1*, Nasopharyngeal Carcinoma WHO Type 3, advanced stadium (III and IV)

INTRODUCTION

Nasopharyngeal carcinoma is as endemic healthy which is often happen in some countries. In indonesia, there are 13,000 new cases with the prevalent of 6.2 per-100,000 population in General Hospital of Dr. Moewardi in the period of 2008 - 2009, Nasopharyngeal Carcinoma WHO Type 3 was reported in amount of 81.9% [1], however, in the period of 2012 - 2014, there was 298 new cases. Data was collected from medical record and specimen of patology anatomy laboratory examination of Dr. Moewardi General Hospital expressed that nasopharyngeal carcinoma was obtained on *fossa rossenmuler* and Spreading to intracranial with the complain of hearing decline, tinitus, difficult to swallow, and eye pain [2]. Early detection of nasopharyngeal carcinoma sufferers are caused by clinic illustration and non-specific serology observation [3].

Protein of *Trombospondin-1* (TSP-1) in preventing the angiogenesis on nasopharyngeal carcinoma sufferers, *Trombospondin-1* is as *glycoprotein* which is synthesized over the induction of P53 which can be used as the detection and specification of nasopharyngeal carcinoma [4][5]. Gen of P53 is functioned as the tumor suppressor by inhibiting the angio-genesis through the activation of TSP-1. However, on nasopharyngeal carcinoma type 3 is reported that P53 is bind with EBNA-LP, so the function of P53 is hampered. There is also reported that EBNA-LP inhibits the signal of *B-Cell-Receptor* (BCR) and maintains the infection of *Eipstein Barr Virus* (EBV) in the condition of latent and lytic phase [6]. The expression of P53 is related with the recurrence of tumor, number of disease free, and life endurance. Tumor recurrence causes the worse prognostic and it increases the number of patient dead of nasopharyngeal carcinoma [7]. However, the expression of P53 mutant on the malignancy of neck head indicates the resistance to the radiotherapy and chemotherapy [8].

Trombospondin-1 is as the cellular matrix of glycol protein which is produced when the physiologic condition by an amount of normal cell [9]. The extra cellular matrix like *fibronectin* can be bind with TSP-1 on the cell surface, the bond of CD 36 with TSP-1 is functioned as anti angio-genesis [10][11][9]. Angiogenesis is as one of the important factors on cancer cell which can be developed, however, TSP-1 is role in regulating the angiogenesis. On the early stadium of cancer, the content of TSP-1 is high. If the exposure is longer, it can cause the hypoxia, it increases the secresi of Vascular Endothelia Growth Factor (VEGF) of tumor and causes the angio-genesis [12][13]. The high concentration of TSP-1 is activating the plasmin so the tumor cell can invasion surrounding it [14]. The role of TSP-1 is also known to be able to activate the Transforming Growth Factor-B (TGF-B) which is role in functioning the physiologic function like the angio-genesis inhibitor and wound healing, proliferasisel, matrix formation of extra cellular, and imun respon [14][9].

MATERIAL AND METHOD

Collected data is come from medical record and observation speciment of patology anatomy laboratory on Dr Moewardi General Hospital for the periode of January 2012 until December 2014. There are obtained the nasopharyngeal carcinoma in amount of 298 persons with the nasopharyngeal carcinoma WHO-type-1 in amount of 9 persons, type-2 in amount of 32 persons, and type-3 in amount of 257 persons. Sufferers with nasopharyngeal carcinoma WHO type-3 due to the consercutively sampling are in amount of 24 persons and there are carried out the observation of P53 and *trombospondin-1*. The research design is observational

analytic by crossectional research design of 24 samples and it is obtained as 12 persons of stadium III and 12 persons of stadium IV.

Clinic level of nasopharyngeal carcinoma is observed based on the TNM (Tumor, Limfonodi, Metastasis) according to AJCC 2002. Biopsi is collected from the patient under supervision and management by Department of Otorhinolaringology Head & Neck Surgery on Dr. Moewardi General Hospital. Before the activity as above, the subject is given information that the consent and observation have been recognized by the ethic committee of Dr. Moewardi General Hospiral/ Medical Faculty Sebelas Maret University. Result of biopsi is inserted into PBS (Phosphat Buffer Saline) and it is directly sent to the patology anatomy laboratory of Dr. Moewardi General Hospital for histopatology observation by painting the Haematocillin Eosin (HE). However, the result of patology anatomy WHO type 3 is sent to the patology anatomy laboratory of Sebelas Maret University for the observation of IHC.

Painting of IHC

Parafin block is cut as the width of 4 micron is put on the slide of poli-L-lysine glass slide (sigma). It is carried out deparafinisation retrieval antigent which is carried out on the microwave oven by buffer citrate with pH of 6.4. Then the slides are inserted into metanol H_2O_2 of 0.3% during 30 minutes. After that, it is washed by Phosphat Buffer Saline (PBS) with the pH of 7.0 in phenol solution of 10%. In the end, it is carried out the humified chamber blocking reagent during 30 minutes and then it is washed by PBS. In sequencely, it is added the primary antibody. The next step is to save it in refrigerator during 18 hours and then it is washed by PBS and it is added by secondary antibody universal (trekkie antibody) with biotine label on the temperature of 30^o C , then it is washed by PBS. The next step is to paint it by caunterstein. The result is read by the expert staf of patology anatomy of Medical Faculty on Sebelas Maret University. Research of histologic score uses the olysia software.

RESULT AND DISCUSSION

Result

Study of population

Table 1 presents the characteristic of nasopharyngeal carcinoma on 2012 until 2014, data from medical record and observation speciment of patology anatomy on Dr. Moewardi General Hospital.

Table 1 The characteristic of nasopharyngeal carcinoma on 2012 until 2014,

Region	Surakarta	Sukoharjo	Karang Anyar	Boyolali	Sragen	Wonogiri	Klaten
Number of population	552,650	863,693	840,171	951,817	896,201	942,377	1,303,910
Male	273,038	428,159	424,597	468,693	444,003	458,090	646,335
Female	279,612	435,534	415,574	483,124	452,198	484,287	670,572
KNF	14 (3.2%)	114 (26.2%)	50 (11.5%)	67 (15.4%)	92 (21.1%)	62 (14.2%)	35 (8%)
Male	11	86	26	49	80	46	26
Female	3	28	24	18	12	16	9
Age of KNF sufferer							
21-30	1 (7.1%)	5 (4.3%)	2 (4%)	3 (4.4%)	3 (3.2%)	1 (1.6%)	1 (2.8%)
31-40	7 (50%)	10 (8. 7%)	4 (8%)	7 (10.4%)	17 (18.4%)	7 (11.3%)	6 (17.1%)
41-50	2 (14.3%)	25 (22%)	7 (14%)	16 (23.8%)	18 (19.5%)	21 (33.8%)	5 (14.2%)
51-60	2 (14.3%)	58 (50.8%)	17 (34%)	33 (49.2%)	18 (19.5%)	39 (62.9%)	15 (42.8%)
> 60	2 (14.3%)	15 (13.1%)	13 (26%)	11 (16.4%)	16 (17.3%)	20 (32.2%)	8 (22.8%)
Job							
Public staf	2 (14.2%)	7 (6.1%)	5 (10%)	7(10.4%)	5 (5.4%)	7 (11.3%)	3 (8.5%)
State staf	2 (14.2%)	11 (9.6%)	4 (8%)	8 (11.9%)	6 (6.5%)	8 (12.9%)	6 (17.1%)
Entepreneur	9 (64.2%)	22 (19.2%)	9 (18%)	16 (23.8%)	22 (23.9%)	15 (24.2%)	9 (25.7%)
Farmer	1 (7.1%)	72 (63.1%)	31 (62%)	36 (53.7%)	39 (42.4%)	53 (85.4%)	17 (48.5%)
Education							
Elementary	1 (7%)	72 (63.1%)	33 (66%)	37 (55.2%)	49 (53.2%)	31 (50%)	16 (457%)
Junior high school	8 (57.1%)	26 (22.8%)	8 (16%)	16 (23.8%)	31 (33.6%)	16 (25.8%)	10 (28.5%)
Senior high school	4 (28.5%)	11 (9.6%)	4 (8%)	8 (11.9%)	7 (7.6%)	8 (12.9%)	6 (17.2%)
University	1 (7%)	5 (4.3%)	5 (10%)	6 (8,9%)	5 (5.4%)	7 (11.2%)	3 (8.5%)
Tiype of Histopathologyy							
I	0 (0%)	1 (0.87%)	1 (2%)	1 (1.4%)	3 (3.2%)	1 (1. 6%)	2 (5.7%)
II	2 (14.2%)	12 (10.5%)	7 (14%)	10 (14.9%)	4 (4.3%)	5 (8%)	2 (5.7%)
III	12 (85.7%)	101 (88.5%)	42 (84%)	56 (83.5%)	85 (92.4%)	56 (90.3%)	31 (88.5%)

Figure 1 presents the distribution of nasopharyngeal carcinoma sufferer per-region who go for the treatment on the Otorinolaryngology polyclinic of Dr Moewardi General Hospital on the period 2012 until 2014

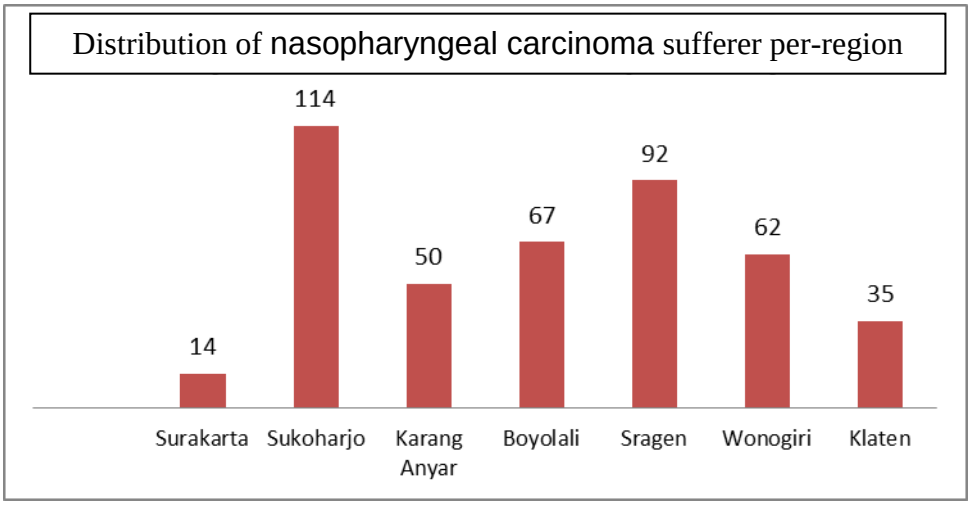

Figure 1. Distribution of nasopharyngeal carcinoma sufferer per-region

Description of sample characteristic

Number of samples on nasopharyngeal carcinoma WHO type 3 in this research are 24 samples which are expressed in the form of frequency distribution and it is presented as in Table 2.

Table 2. Description of sample characteristic

Characteristic	Frequency (%)	Stadium III (n = 12)	Frequency (%)	Stadium IV (n = 12)	Frequency (%)
Age (year):					
30 – 40	3 (12.5)		2 (16.7)		1 (8.3)
41 – 50	6 (25.0)		3 (25.0)		3 (25.0)
51 – 60	8 (33.3)		3 (25.0)		5 (4.7)
> 60	7 (29.2)		4 (33.3)		3 (25.0)
Sex:					
Male	18 (75.0)		18 (75.0)		8 (66.7)
Female	6 (25.0)		6 (25.0)		4 (33.3)

Table 2 presents the sample distribution based on the category of sample characteristic. The most age of 24 samples is in the range of 51 - 60 years old, however, males are more than females.

Description of P53 expression and *Trombospondin-1* is expressed in mean and median and it is presented as in Table 3.

Table 3. Description of P53 expression and *Trombospondin-1*

Variable	Description value
P53	
Mean ± SD	7.66 ± 0.95
Median (range)	7.56 (6.11 – 9.44)
Trombospondin-1	
Mean ± SD	7.78 ± 1.21
Median (range)	7.50 (6.11 – 9.98)

Table 3. presents the description value on histhology score of P53 expression and *Trombospondin-1* in the form of mean and median. The linearity test (p) is 0.163 (p > 0.05). It means that there is no significant difference between P53 expression and *Trombospondin-1*.

Linear correlation test (Pearson's product moment) indicates that the histhology score of P53 sekresi and *Trombospondin-1* produces the correlation (r) is 0.473 and (p) = 0.020 (p < 0,05). Histhology score of P53 and *Trombospondin-1* which is categorily expressed can be seen as in Table 4.

Table 4. Category description of P53 expression and *Trombospondin-1*

Variable	Frequency (%)
P53	
Soft positive	12 (50,0)
Moderate positive	12 (50,0)
Trombospondin-1	
Soft positive	12 (50.0)
Moderate positive	12 (50.0)

Table 4. presents the P53 expression and *Trombospondin*-1. Each of samples consist of 12 persons (50%) and each of them express the soft and moderate positive (50%) and it can be seen as in Figure 2.

Table 5. Category description of TSP-1 expression

TSP-1	Stadium III (n = 12)	Stadium IV (n =12)
Soft positive	4 (33.3)	8 (66.7)
Moderate positive	8 (66.7)	4 (33.3)

Table 5. presents the category distribution difference of TSP-1 expression between sample of stadium-III and stadium-IV.

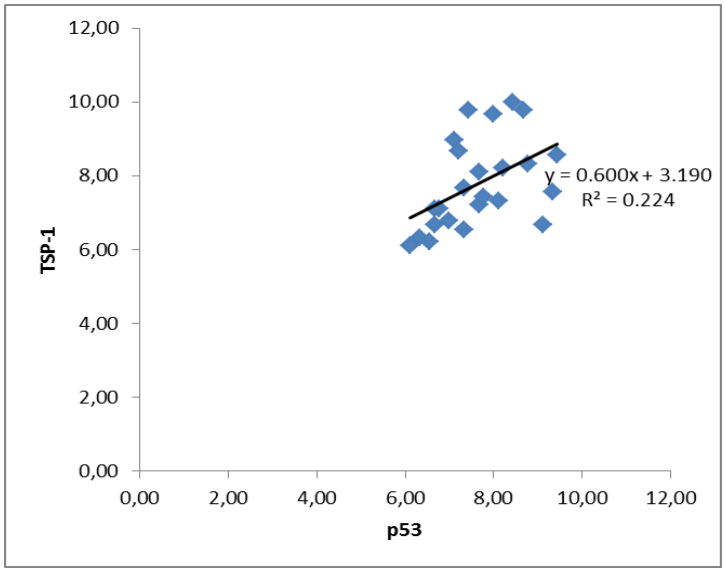

Figure 2. Scatter diagram of the relation between histhology score of P53 expression and *trombospondin-1*

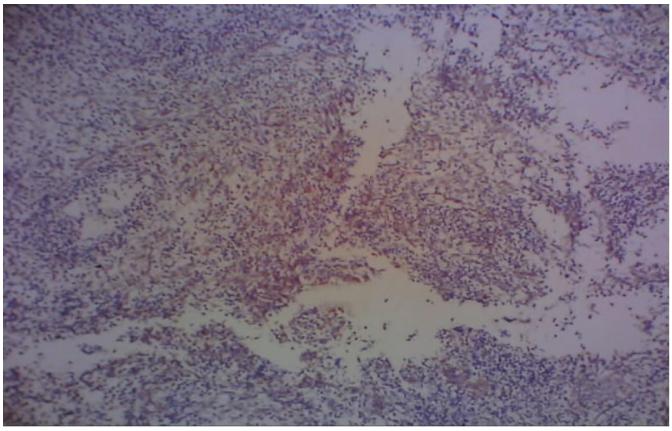

Figure 3. P53 expression on the nasopharyngeal carcinoma with enlargement of 100x by olymphus CX.21 microscop

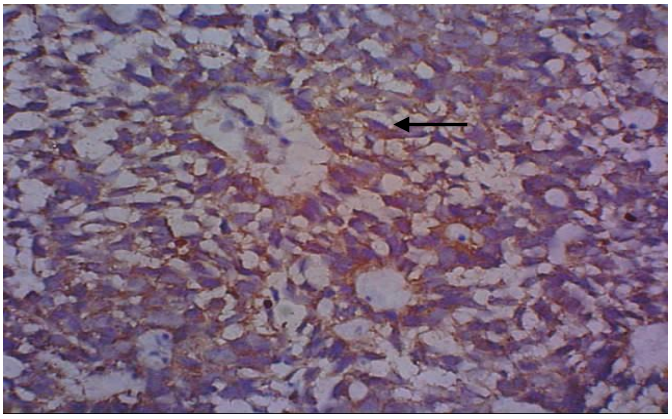

Figure 4. P53 expression on the nasopharyngeal carcinoma with enlargement of 400x by olymphus CX.21 microscop

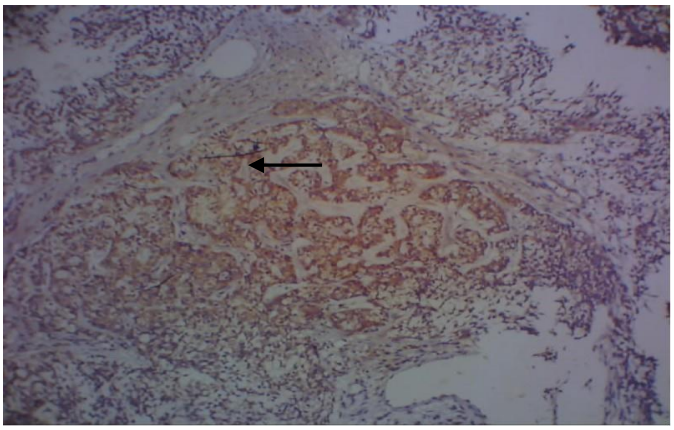

Figure 5 *Trombospondin-1* expression on the carsinoma naso-faring with enlargement of 100x by olymphus CX.21 microscop

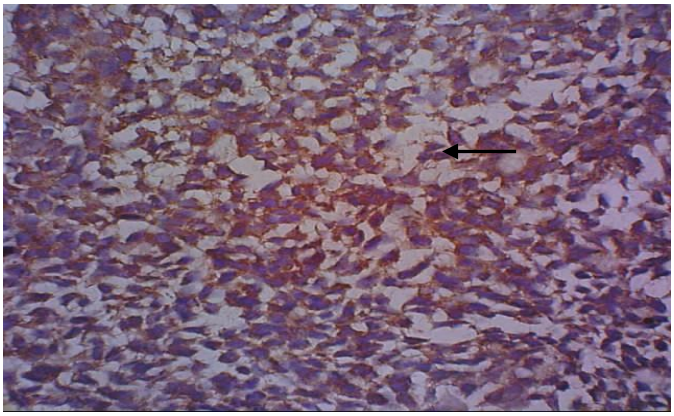

Figure 6. *Trombospondin-1* expression on the nasopharyngeal carcinoma with enlargement of 400x by olymphus CX.21 microscop

Table 6. Difference test on the histhology score of TSP-1 expression between ample of Stadium III and Stadium IV

TSP-1	Stadium III (n = 12)	Stadium IV (n = 12)	p
Mean ± SD	8.27 ± 1.24	7.30 ± 1.00	0.045
Median (range)	8.38 (6.33 – 9.98)	7.16 (6.11 – 9.78)	

Table 6. presents the menu different test of Whitney Test that produces the pj is 0.045 and p < 0.05). It menas that TSP-1 expression on patient of stadium-III is higher that stadium-IV.

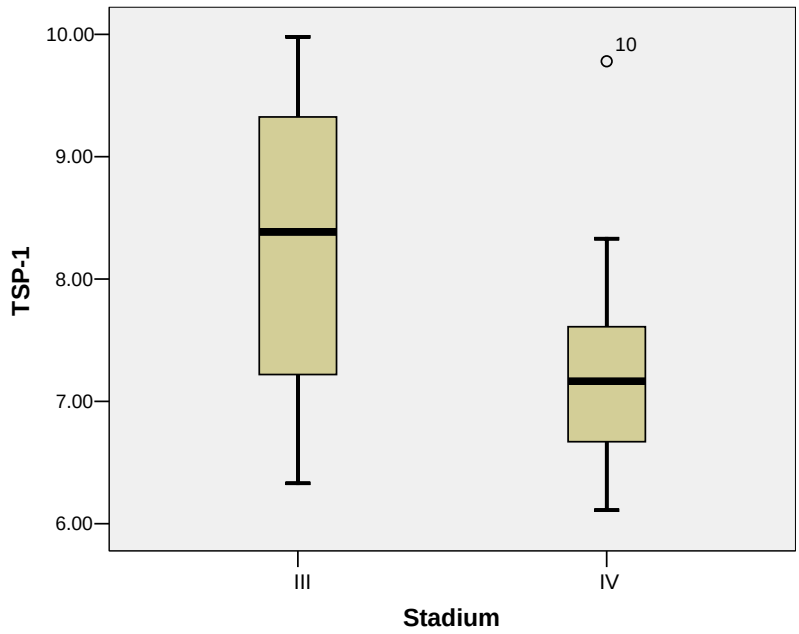

Figure 7. Boxplot on histhology score comparison of TSP-1 expression between Sample of Stadium-III and IV

DISCUSSION

Collected result from medical record and speciment observation of patology anatomy from 2012 until 2014. nasopharyngeal carcinoma sufferer who have treatment in Otorhinology polyclinic of Dr. Moewardi General Hospital are 298 persons with the WHO type 3 are 257 persons, WHO type 2 are 232 persons, and WHO type 1 are 9 persons. Based on the data as above, we carried out the research of P53 and Trombospondin-1 on the nasopharyngeal carcinoma WHO type-3. This research is as observational research with the

method of consecutive sampling of 24 nasopharyngeal carcinoma samples which intends to prove the relation between P53 expression with TSP-1 nasopharyngeal carcinoma WHO type-3 in Dr. Moewardi General Hospital.

Based on the age, the most age is obtained in the range of 51-60 years old (33.3%). It indicates that there is needed a long time for processing the inisation by antigent until the happening of tumor.

Based on the sex, the result shows that male (75%) is more than female (25%), however, Brennan [15] reported as the ratio is 3 : 1. It is caused that male has the risk factor for the hapenning of tumor such as peptiside expossed, smoking, and the other factor of carsi-genesis.

Hispathology score result of P53 expression is 7.66, but TSP-1 is 7.78 with the linear correlation test (Pearson's product moment) as follow: the correlation coefficient is 0.473 with the probability (p) is 0.02 ($p < 0.05$). It indicates that there is the significant relation with moderate strength[16]. It is regarding with the theory of P53 which inducts the TSP-1 which will inhibit the happening of angiogenesis [6]. The role of P53 in tumor cell is as the tumor superior protein through the increasing of apoptosis, stopping the cell cycle, rapairing the DNA, and inhibiting the angiogenesis [17] which the increasing of apoptosis can be through extrinsik line by activating caspase 8 as well as instrinsik line throught direct activation [18].

The role of P53 is to repair DNA by inducting NER of DNA and recombining chromosome [19], in stopping the cell cycle is by inhibiting the cdk and p21 [20]. The role of P53 in inhibiting the angi-genesis is through the induction of TSP-1 which is as the important factor in regulating the angio-genesis [14]. Research of Chang [21] produced the mutation of P53 prevalensi on the nasopharyngeal carcinoma was 14%.

This research result produced the positive relation between P53 and *Trombospondin-1*, and there is the strong effect on the low TSP-1 expression on the low level of TSP-1 expression.

CONCLUSION

Based on the analysis as above, it is concluded that the decreasing of TSP-1 expression influences the development of tumor cells on the nasopharyngeal carcinoma WHO type-3.

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