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Role of P27^{kip1} Protein, P45^{skp2} Coactivator, and P38^{jab1} Coactivator in Preventing Rhabdomyosarcoma (RMS) in Oral Cavity of Children

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Abstract

Rhabdomyosarcoma is a rare malignant tumor that attacks the differentiation of skeletal muscle and usually affects children, contributing to about 60% of all soft tissue sarcomas. The purpose of this study was to examine the relationship between p27^{Kip1} immunoexpression and p45^{Skp2} and p38^{Jab1} coactivators, as well as the relationship between p27^{Kip1} immunoexpression and p45^{Skp2} and p38^{Jab1} coactivators on stages and prognosis of oral RMS in children. This was a restrospective study on the immunoexpression of p27^{Kip1} and p45^{Skp2} and p38^{Jab1} coactivators on RMS cells. The RMS stage was determined according to the American Joint Committee of Cancer/AJCC of stages 1–4, and were divided into group I (stages 1 and 2) and group II (3 and 4). Samples were retrieved fromt he paraffin blocks of patients with embryonal RMS. Each paraffin block was cut, and 6 samples with 5 µm thickness from each block were examined using p27^{Kip1}, p45^{Skp2}, and p38^{Jab1} proteins. The analysis was performed using a linear regression test on the relationship between p27^{Kip1} and p45^{Skp2} and p38^{Jab1}, resulting in a p-value of 0.000 (<0.05) and a coefficient value of b -1.36. Meanwhile, the stage was analyzed using the Wald test of 8.0688, resulting in a p-value of 0.0045 with a significant negative correlation. Analysis on the relationship between p45^{Skp2} and p38^{Jab1} and the RMS stage was performed using the Gamma test, resulting in a significant positive correlation (p<0.05).

Keywords: Immunohistochemicals, p45^{Skp2} coactivator, p38^{Jab1} coactivator, p27^{Kip1} protein, RMS

Introduction

Rhabdomyosarcoma is a malignant tumor that attacks the differentiation of skeletal muscle cells, this tumor is rare. Usually affects children, about 60% of all soft tissue sarcomas. While in adults, about 2–5% of all soft tissue sarcomas.[1] Rhabdomyosarcoma usually occurs in the first decade of life but can also occur in adolescence or adulthood, but rarely over 45 years. Embryonic rhabdomyosarcoma occurs mostly in the first 10 years of life and accounts for 60% of all cases.

Uncontrolled cell proliferation is the main characteristic of tumors. Disorders of the cell cycle and an unbalance between cell proliferation and death due to various causes play a crucial role in tumorigenesis and tumor progression. p27^{Kip1}, a negative cell cycle regulator, is a universal cyclin-dependent kinase (CDK) inhibitor (CKI) that belongs to the Cip/Kip group of CDK inhibitors. p27 shares a sequence homology with p21 and p57 and it may bind to and inhibit the activity of cyclin-CDK complexes. Due to the inhibition of p27^{Kip1}, cyclin-CDK cannot effectively phosphorylate the retinoblastoma protein; thus, E2F transcription factors cannot be released, downstream genes cannot be transcribed and the cell cycle process is blocked.[2,3] As a well-recognized tumor suppressor gene, it is able to control cell cycle transition from G1 to S phase. Thus far, various cell cycle regulators have been identified, including the tumor suppressor p27^{kip1}, wafl p21, cyclin A and p53, all of which may be degraded via the Skp2-mediated ubiquitin-proteasome pathway.[4, 5] Thus, Skp2 is key in the p27^{kip1} ubiquitin degradation pathway and may inhibit the proliferation of a variety of cell types through the ubiquitin-proteasome pathway.[6, 7, 8]

Jab1 has been involved to the tumorigenic process, therefore, jab1 have the potential to be an effective beneficial target that affect the interaction of many tumor stage. Size of Jab1 is an -40 kDa soluble protein, also Jab1 is located on chromosome 8. Jab1 interacted with p27^{kip1} and improved its cytoplasmic translocation which resulted in acceleration of p27^{kip1} degradation through the ubiquitin and proteasome pathway.

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It is well known that p27^{kip1} protein levels are mainly regulated through degradation by ubiquit independent proteolysis. Control of p27^{kip1} and Jab1 is important process in cancer progression, which is controlled by both the positive and negative regulators. p27^{kip1} is a kind of cdk inhibitors, suppresses the G1-to-S cell cycle progression, functions as a main negative regulator of apoptosis and is, thus, considered a tumor suppressor.[9] The main function of this p27^{kip1} is to inhibit the CDK2-cyclin E complex by controlling at the G1 to S transition in normal cells.[10,11]

Until now, the incidence of this disease is increasing and many are detected at an advanced stage so that there is a delay in diagnosis due to the gap between clinical and pathological examinations in determining the stage and prognosis. Early detection of RMS is very important for the treatment of cancer.

Methods

This type of research is a clinicopathological study with the variable component used is an immunohistochemical assessment of the expression of p27^{Kip1} protein and its coactivators (p45^{Skp2} and p38^{Jab1}) in oral RMS tumor tissue cells in children. The method was carried out retrospectively by examining the immunoexpression of p27^{Kip1} and the coactivators p45^{Skp2} and p38^{Jab1} on RMS cells. Samples were taken through the technique of completeness and feasibility criteria for the RMS tissue preparation and the completeness of the medical record book. From the book, records of age, gender, clinical information, clinical diagnosis, histopathological diagnosis, and clinical stage were obtained. The representative paraffin block samples were then cut using a microtome and 7 new preparations were made. 1 preparation for staining hematoxylin eosin to reassess histopathological diagnosis, 2 preparations for staining p27^{Kip1} immunoexpression and 4 preparations for staining immunoexpression of its coactivator. The method of data collection is based on cross sectional. For data analysis: 1) Testing the size of the sample by calculating the power of the test; 2) Testing the relationship between p27^{Kip1} immunoexpression with coactivators p45^{skp2} and p38^{Jab1} was carried out by testing the regression coefficient between each variable; 3) Testing the relationship between p45^{Skp2} and p38^{Jab1} with stage using Gamma Correlation to explain the relationship between p45^{Skp2} and p38^{Jab1} with stage. Sampling was in the form of paraffin blocks from the Anatomical Pathology Laboratory of De. Hasan Sadikin General Hospital and Sardjito Hospital UGM Yogyakarta. The place of research was carried out at the Integrated Research Laboratory, Gajah Mada University, Yogyakarta.

Result

The results were obtained from 17 paraffin blocks of embryonal RMS oral tissue for children, 11 paraffin blocks were obtained from the Department of Pathology Anatomical, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung. Six paraffin blocks from Dr. Sardjito General Hospital, Yogyakarta. Based on the number of samples as many as 17 paraffin blocks contained in the data were divided into two groups, namely stage 1 and 2 (group I), stage 3 and 4 (group II).

Based on the data above, it is calculated through the power of the test, the results show that for AUC under H_0 it is 0.5 and AUC under H_1 is 0.82, meaning that for an AUC area difference of 0.32 with an alpha of 0 0.05, the test power of 0.7862 was obtained. Table 2 shows the observation of the immunoexpression of p27^{Kip1}, p38^{Jab1}, p45^{Skp2} and stage in patients with embryonal child oral rhabdomyosarcoma.

Table 2. Shows the age distribution of children aged 9 months to 13 years who suffer from oral RMS with variations from stage 1 to 4 which have a mean value of immunoexpression of p27^{Kip1} and the coactivators of p45^{Skp2} and p38^{Jab1} which also vary. When viewed from the distribution of the mean immunoexpression of p27^{Kip1} with the distribution of the average immunoexpression of p45^{Skp2} and p38^{Jab1}, the results were inversely proportional to the lower mean value of immunoexpression of p27^{Kip1}, the higher the mean value of immunoexpression of p45^{Skp2} and p38^{Jab1}. Furthermore, the results of the mean value of immunoexpression of p27^{Kip1} compared to the RMS stage of patients showed the lower the mean value of immunoexpression of p27^{Kip1}, the higher the stage of RMS patients, while the mean values of immunoexpression of p45^{Skp2} and p38^{Jab1} against the stage had a directly

Table 1 Summary of Stadium Data

Stadium (Group)	Total
Stage 1 and 2 (group I)	10
Stage 3 and 4 (group II)	7

Table 2 Observation of the Immunoexpression of p27^{Kip1}, p38^{Jab1}, and p45^{Skp2} Against the Stage in children with Oral RMS

Age (years)	Stadium	P27(1)	P27(2)	X	P45(1)	P45(2)	X	P38(1)	P38(2)	X	Notes
8	1	30	28	29	6	8	7	2	3	2.5	400X
1	1	34	32	33	2	6	4	1	2	1.5	1000X
1	4	5	2	3.5	57	61	59	43	38	40.5	
14	1	45	39	42	1	2	1.5	2	1	1.5	
3	4	5	8	6.5	37	34	35.5	24	21	22.5	
3	1	28	32	30	4	6	5	2	3	2.5	
13	4	7	8	7.5	49	52	50.5	30	28	29	
11	4	2	3	5.2	39	43	41	23	20	21.5	
6	1	31	29	30	3	5	4	4	3	3.5	
7	2	26	30	28	9	6	7.5	5	8	6.5	
6	4	5	7	6	50	40	45	30	20	25	
5	2	28	27	27.5	10	9	9.5	5	7	6	
3	2	8	13	10.5	38	35	36.5	25	22	23.5	
6	4	7	11	9	57	48	52.5	29	30	29.5	
6	3	11	9	9.5	32	39	35.5	29	28	28.5	
9 month	2	24	27	25.5	8	11	9.5	7	9	8	
2	1	30	20	25	7	4	5.5	4	6	5	

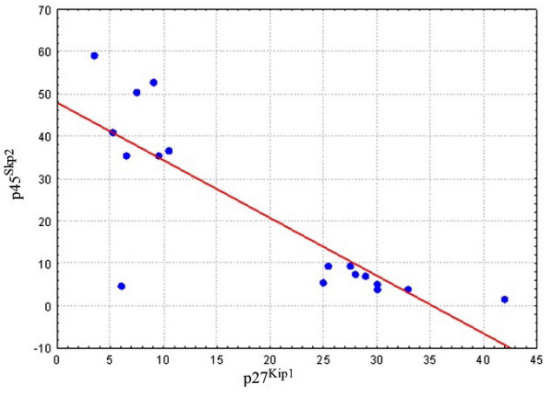

Figure 1 Relationship between p27^{Kip1} and p45^{Skp2}

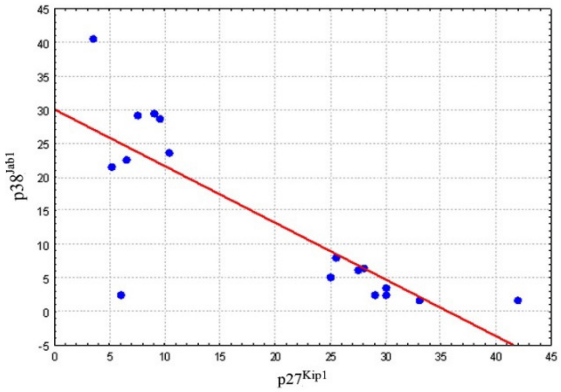

Figure 2 Relationship between p38^{Jab1} Over p27^{Kip1}

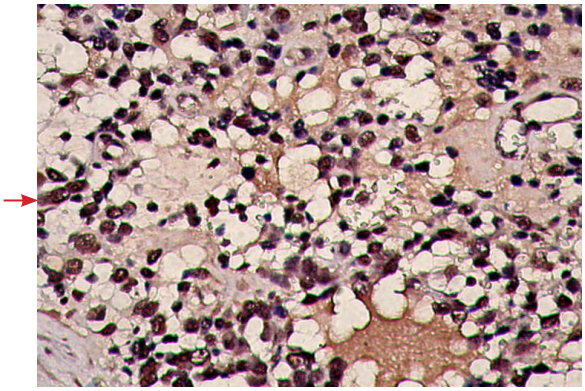

Figure 3 Immunoexpression Distribution of p27^{Kip1} in the Nucleus of Stage 1 Cells

The red arrows indicate the distribution of p27^{Kip1} immunoexpression in the nucleus of cancer cells (brown)

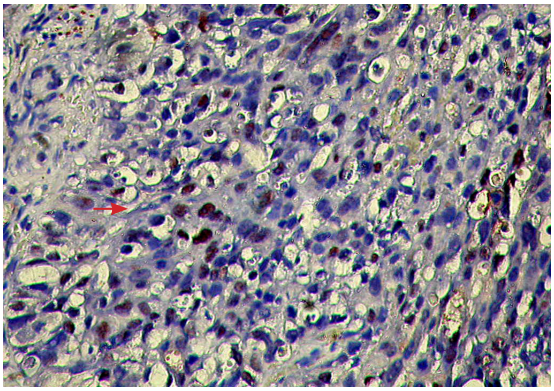

Figure 4 Immunoexpression Distribution of p45^{Skp2} in the Nucleus of Stage 4 Cells

The red arrow indicates one of the distributions of p45^{Skp2} immunoexpression in the nucleus of cancer cells (brown color)

proportional value.

The mean of immunoexpression of the coactivator p45^{Skp2} in the nucleus of the cells had a greater distribution when compared to the immunoexpression of p38^{Jab1}. This is because the expression of p45^{Skp2} has a function and serves as a degrading agent for p27^{Kip1} in the cell nucleus, while p38^{Jab1} in the nucleus only carries p27^{Kip1} which is not degraded in the cell nucleus, and then transported to the cytoplasm. In the cytoplasm, the distribution of p38^{Jab1} became more abundant after the protein broke away from its binding to the phosphorylated p27^{Kip1}. Similar results were reported by Supriatno et al. that p45^{Skp2} is more strongly expressed in the cell nucleus than in the cytoplasm through

Table 3 Regression Coefficient Testing p45^{Skp2} Over p27^{Kip1}

	B	SE (B)	t (15)	p-level
Intercept	47.94	5.539	8.66	0.000
"p27^{Kip1}"	-1.36	0.244	-5.59	0.000

Table 4 Regression Coefficient Testing p38^{Jab1} Over p27^{Kip1}

	B	SE (B)	t (15)	p-level
Intercept	30.05	3.579	8.40	0.000
p27^{Kip1}	-0.84	0.158	-5.35	0.000

immunohistochemical analysis.

The following Figure 5 shows one of the results of the immunoexpression of the p27^{Kip1} protein, p45^{Skp2} coactivator, p38^{Jab1} that occurs in the cell nucleus and cytoplasm. The results of the brown stain on the nucleus of cancer cells indicate the distribution of p27^{Kip1} immunoexpression.

Discussion

Rhabdomyosarcoma is a malignant tumor that spreads by direct extension, lymphatic metastases, and hematogenous metastases.[12] About 15% of rhabdomyosarcomas have metastases at the time of diagnosis.

Discussion Rhabdomyosarcoma is the third most common extracranial malignant tumor in children. The highest incidence of rhabdomyosarcoma is in children aged 1–4 years, the lowest incidence is at the age of 10–14 years, and the lowest is in the age group of 15–19 years or young adults.[13,14] About 35% of rhabdomyosarcomas occur in the head region. and neck. Rhabdomyosarcoma in the oral cavity is rare.[14,15]

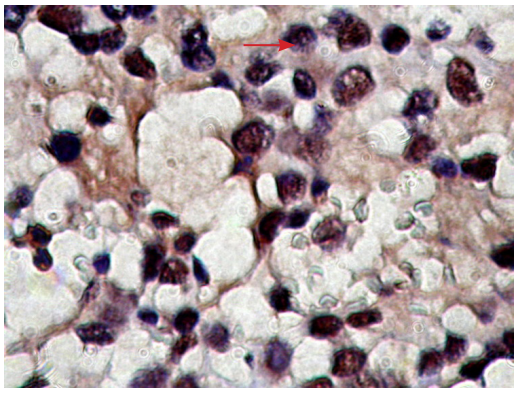

Figure 5 Distribution of p38^{Jab1} Immunoexpression in Stage 4 Cytoplasm

The red arrow indicates one of the distributions of p38^{Jab1} immunoexpression in the cytoplasm (brown color)

The calculation of the correlation between p27^{Kip1} immunoexpression and p45^{Skp2} and p38^{Jab1} immunoexpression is shown in Table 3.

In the p-level column, it is known that the p value is 0.000 (<0.05), besides the coefficient value of b=-1.36 (negative) which is smaller than F table 99%. This means that there is a negative and significant relationship between p27^{Kip1} and p45^{Skp2}.

The same results as Table 3. are shown in Table 4. that between p27^{Kip1} and p38^{Jab1} In the p-level column it is known that the p-value is 0.000 (<0.05), besides the coefficient b=-0.84 (negative) which is higher smaller than F table 99%. This indicates that there is a negative and significant relationship between p27^{Kip1} and p38^{Jab1}. The test results showed a decrease in p27^{Kip1} immunoexpression led to an increase in oral RMS p45^{Skp2} and p38^{Jab1} immunoexpression in children.

The immunoexpression relationship of coactivators p45^{Skp2} and p38^{Jab1} using Gamma correlation to explain the relationship between p38^{Jab1} and p45^{Skp2} with stage gave the result that the relationship between p45^{Skp2} and stage was positive, meaning that the higher the level of p45^{Skp2}, the higher the stage and the relationship between the two was significant (p value <0.05). The same is true for the relationship between p38^{Jab1} and stage; the relationship was positive and significant (p value <0.05). The same is true for the relationship between p38^{Jab1} and stage; the relationship was positive and significant (p<0.05).

Several studies state that the expression of p27^{Kip1}, Jab1, and Skp2 provided a clinical reference for the treatment of Non-Hodgkin lymphoma (NHL).[16] The correlation between p27^{Kip1} expression and other tumor suppressor genes may turn out to be important in determining the prognosis of Head and Neck Squamous Cell Carcinoma (HNSCC).[17]

Kim et al.[18] stated that Jab1 is a protein that enhances the activity of the c-jun gene and induces cell proliferation, p27^{kip1} is a strong reduction in the tumor suppressor gene p27^{kip1} has a high resistance and poor prognosis in

cancer.Also, p27^{kip1} has an important role in cell cycle regulatory factors. Recently, Jab1and p27^{kip1} has been reported that many women are involved in diseases such as breast cancer, endometriosis, and ovarian cancer. As well as Jab1 and p27^{kip1} started to reveal that the association became known widely in hepatocellular carcinoma, laryngeal carcinoma, and nerves.[19]

Other investigators suggest that Skp2 may degrade p27^{kip1} protein through the ubiquitin-proteasome pathway, thus, participating in the occurrence and development of hypopharyngeal squamous cell carcinoma. Furthermore, the abnormal protein expressions Skp2 and p27^{kip1} appear to be associated with the poor prognosis of hypopharyngeal squamous cell carcinoma. Therefore, combined detection of Skp2 and p27^{kip1} may provide significant guidance for comprehensively determining the malignant degree and prognosis of patients with hypopharyngeal squamous cell carcinoma.[19]

The diagnosis is confirmed by an incisional biopsy in the center of the tumor. Radiographs, CT scans, or MRIs are needed to determine tumor size, anatomical spatial relationships, and extent of bone damage. CT scans and MRIs should record detailed skull base sections to assess for potential invasion into this area and cover the entire skull for metastases or extensions to the brain. A PET scan is necessary to look for distant metastases, and because rhabdomyosarcoma tends to metastasize to the bone marrow, bone marrow aspiration is also recommended.[20]

Generally, cancers located in the oral cavity will metastasize to regional lymph nodes, especially in the front of the neck, then form spreads to the lungs, liver, and bones. Therefore, if a child with RMS who already has an advanced stage is found to have symptoms of respiratory problems and swelling of the heart. In addition to metastasizing, RMS at an advanced stage can also invade deeper tissue structures and penetrate the basement membrane, which is known as RMS cell invasion or migration.[21]

Rhabdomyosarcoma in the oral cavity is more common in men than in women and mostly occurs in the second decade of life.[14,22] Life expectancy for rhabdomyosarcoma sufferers has increased from year to year. In 1975-1979 the life expectancy of patients was 49% and in 2003-2009 it reached 64%.[23]

Research has shown an inverse relationship between p27^{Kip1} immunoexpression with the p45^{Skp2} and p38^{Jab1} oral RMS coactivators in children. The increase in staging was indicated by a decrease in the immunoexpression of p27^{Kip1} and an increase in the immunoexpression of the coactivators p45^{Skp2} and p38J^{ab1}, so that examination of the series of relationships between the immunoexpression of p27^{Kip1} and the immunoexpression of the coactivators p45^{Skp2} and p38^{Jab1} with staging could be used to support the prognosis of oral RMS in children.

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