Research Article

KRAS and BRAF Mutation in Borderline Epithelial Type Ovarian Tumor

Mutasi KRAS dan BRAF pada Tumor Ovarium Tipe Epitel Borderline

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Abstract

Objective: To understand the molecular profile by identifying the mutation of KRAS and BRAF in borderline type ovarian tumor.

Method: In the study, we examined paraffin tissue sample from Department of Pathology Anatomy, University of Indonesia/Dr. Cipto Mangunkususmo Hospital, Jakarta, that was diagnosed as borderline epithelial ovarian tumor. Seventeen samples were taken to Sandya Laboratory in Bandung for examination of PCR BRAF exon 15 codon 600, and KRAS in exon 2 codon 12 and 13, as well as exon 3 codon 61.

Result: Mutation of KRAS occurred in 94% of subjects (serous borderline 62.5%, mucinous bordeline 37.5%), of which 70.6% mutation happened in exon 2 codon 12 (serous borderline 33.3%, mucinous borderline 66.7%), 52.9% mutation in exon 2 codon 13 (serous borderline 33.3%, mucinous borderline 66.7%), and 76.5% mutation in exon 3 codon 61 (serous borderline 30.8%, mucinous borderline 69.2%). Mutation of BRAF occurred only in 47% of subjects, but the results of Exact Fisher test showed that mutation in BRAF gave significant result, while other variables did not give significant result (p=0.009).

Conclusion: Molecular pathology in borderline ovarian tumor related with BRAF mutation is more likely to occur in serous borderline type, while KRAS mutation is more likely to occur in mucinous borderline type.

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Keywords: borderline ovarian tumor, BRAF, KRAS

Abstrak

***Tujuan**: Untuk mengetahui mutasi KRAS dan BRAF pada tumor ovarium type borderline.*

***Metode**: Bahan penelitian adalah blok parafin jaringan yang berada di Bagian Patologi Anatomi, FKUI/RSUPNCM Jakarta yang telah didiagnosis sebagai tumor ovarium tipe borderline. Tujuh belas sampel dibawa ke Laborataorium Sandya di kota Bandung untuk dilakukan pemeriksaan PCR BRAF Exon 15 Codon 600, dan KRAS pada Exon 2 codon 12 dan 13, serta exon 3 codon 61.*

***Hasil**: Mutasi KRAS terjadi pada 94% sediaan (borderline serosum 62,5%, borderline musinosum 37,5%), dengan perincian mutasi pada exon 2 codon 12 sebanyak 70,6% (borderline serosum 33,3%, borderline musinosum 66,7%), mutasi exon 2 codon 13 sebanyak 52,9% (borderline serosum 33,3%, borderline musinosum 66,7%), dan mutasi pada exon 3 codon 61 sebanyak 76,5% (borderline serosum 30,8%, borderline musinosum 69,2%). Mutasi BRAF terjadi hanya pada 47% sediaan, tetapi hasil uji Eksak Fisher memperlihatkan bahwa mutasi pada BRAF memperlihatkan hasil signifikan, sementara variabel yang lainnya tidak memberikan hasil yang signifikan (p=0,009).*

***Kesimpulan**: Tampak adanya kecenderungan pada tipe borderline serosum lebih banyak terjadi mutasi pada BRAF, sedangkan pada tipe borderline musinosum lebih banyak terjadi mutasi KRAS.*

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***Kata kunci**: BRAF, KRAS, tumor ovarium borderline*

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INTRODUCTION

Intra-abdominal tumor is mostly associated with ovarian malignancy. It is found in approximately 8% of all gynecological malignant tumor at all age, but 60% is found in the women of more than 50 years old, 30% in reproductive age, while the remaining is found in younger age.[1,2] Recently, it is expected that there have been more cases with histopatological characteristic between benign ovarian tumor and malignant ovarian tumor, which is classified as borderline ovarian tumor, the treatment of which has not been fully agreed on. It is expected that approximately 9.2-20% of whole ovarian malignancy is this type of tumor, with the survival rate of 95% despite the fact that the recurrence and mortality may occur in 10 to 20 years later. This is because this type of tumor has the ability to metastasize to the organs far beyond the internal genitalia. In Indonesia, ovarian malignant tumor is commonly found and is the third largest cause of death after cervical malignant tumor and breast cancer, while the five-years survival rate of 20-37%.[1]

Identification and knowledge about the characteristics of molecular change associated with carcinogenesis will facilitate the development of diagnostic test to detect early ovarian carcinoma and to develop specific therapy that could block or minimize the signals of tumor growth.[3]

Until now the pathogenesis of ovarian carcinoma is unknown. Likewise is the progressivity.[4] Ovarian carcinoma is very heterogeneous, and primary it is classified on the basis of cellular types into serous, mucinous, endometrioid, clear cell, and Brenner (transitional). It is associated with the difference of epithelial types of women's reproduction organ.[5,6]

Tumors in this category is further divided into 3 (three) groups, which are benign, malignant and intermediate (borderline tumor), reflecting the characteristics of the carcinoma. Borderline tumor of mucinous and endometrioid type are frequently associated with invasive carcinoma, but borderline serous tumor is seldom associated with serous carcinoma.[5] In further observation, the advancement of molecular genetics studies has shown differences of frequency of mutation of p53 and KRAS in serous carcinoma and in borderline tumor of serous. This has made researchers conclude that probably serous borderline tumor is not related to serous carcinoma.[5-8] The unspecific characteristics of borderline tumor are reflected in the word borderline it self, implying ambiguous condition. Based on the review of molecular and clinical pathology studies, a model of tumor development is proposed. In this model, epithelial tumor deriving from ovarian surface is divided into two large categories: type I and type II based on the characteristics of the pathogenesis. Type I tumors tend to be low-grade tumor growing from borderline tumors, while type II tumors are high grade tumors where the precursor lesion is morphologically unidentified and thus is called to have de novo development.[9]

These carcinogenesis model shows the correlation between borderline tumors and invasive carcinoma and describes morphological and molecular structure that can be used to clarify the pathogenesis of ovarian cancer.[9]

There are some specific molecular changes that differentiate low grade and high grade carcinomas such as the genetic mutation of BRAF and KRAS. Cascade of RAS, RAF, MEK, ERK, and MAP play important role in transmitting growth signals to stem cells. Oncogenic mutation of BRAF and KRAS show activation of this type and help neoplastic transformation process.[7,10-12] Mutation of BRAF and KRAS seem to occur at very early phase of low grade serous carcinoma growth as indicated by the mutations in small atypical proliferative type serous tumor.[13,14]

METHOD

In the study, we examined paraffin tissue sample from Department of Pathology Anatomy, University of Indonesia/Dr.Cipto Mangunkususmo Hospital, Jakarta, that was diagnosed as borderline epithelial ovarian tumor. Seventeen samples were taken to Sandya Laboratory in Bandung for examination of PCR BRAF exon 15 codon 600, and KRAS in exon 2 codon 12 and 13, as well as exon 3 codon 61.

RESULTS

Results of Exact Fisher test show that there is no significant correlation between results of anatomical pathology and patients' characteristics. The results are presented in the following Table 1.

Table 1. Results between Characteristic and PA Examination Results.

Variable	Results of PA		Value p*)
	Serous (n=5)	Mucinous (n=12)	
Age (year):			1.0
• < 40	1	4	
• 40	4	8	
• Standard Deviation (SD)	42.2 (13.9)	42.3 (14.5)	
Parity:			0.319
• 0	3	3	
• 1-3	2	7	
• ≥ 4	0	2	
Menopause Status:			1.0
• Yes	2	6	
• No	3	6	
Solid Part:			0.60
• Yes	4	7	
• No	1	5	
Ascites:			0.131
• Yes	4	4	
• No	1	8	

*Remarks: *) based on Fisher Exact test*

Fisher Exact test results also show that of the five independent variables, only mutation of BRAF shows significant results, while other variables did not give significant result. The results of the test are presented in the following Table 2.

Table 2. Correlation between Mutation of KRAS and BRAF and Results of Histopathology (HP) Examination.

Variable	Results of HP		Value p*)
	Serous (n=5)	Mucinous (n=12)	
Mutation of KRAS Ex2Cod12:			1.0
• Positive	4	8	
• Negative	1	4	
Mutation of KRAS Ex2Cod13:			1.0
• Positive	3	6	
• Negative	2	6	
Mutation of KRAS Ex3Cod61:			1.0
• Positive	4	9	
• Negative	1	3	
Mutation of BRAF Ex15Cod600:			0.009
• Positive	5	3	
• Negative	0	9	

Other Fisher Exact tests to identify the correlation between mutation of BRAF and mutation of KRAS show no significant correlation as presented in the following Table 3.

Table 4. Correlation between RMI Score and Mutation of BRAF and KRAS in Epithelial and Borderline Type Ovarian Tumor.

Variable	Score RMI*)	Value p**)
BRAF Ex15Cod600:		0.321
• Positive (n=8)	391.2 (66.3-9764)	
• Negative (n=9)	203.1 (23.3-2072.8)	
KRAS Ex2Cod12:		0.160
• Positive (n=12)	256.2 (23.3-3104.8)	
• Negative (n=5)	968.0 (203.1-9764)	
KRAS Ex2Cod13:		0.423
• Positive (n=9)	351.6 (23.3-3104.8)	
• Negative (n=8)	339.8 (131.6-9764)	
KRAS Ex3Cod61:		0.871
• Positive (n=13)	351.6 (23.3-9764)	
• Negative (n=4)	549.8 (88.6-3104.8)	

Table 3. Correlation between Mutation of KRAS and BRAF in Epithelial and Borderline Type Ovarian Tumors.

Variable	Mutation of BRAF Ex15Cod600		Value p*)
	Positive (n=8)	Negative (n=9)	
Mutation of KRAS Ex2Cod12:			0.294
• Positive	7	5	
• Negative	1	4	
Mutation of KRAS Ex2Cod13:			0.153
• Positive	6	3	
• Negative	2	6	
Mutation of KRAS Ex3Cod61:			0.576
• Positive	7	6	
• Negative	1	3	

Mann-Whitney test was performed to identify the correlation between the use of RMI score as a method to identify the probability of malignancy and the mutation of BRAF and KRAS show insignificant correlation. But the higher the score of RMI, the more likely the mutation of BRAF was to be found while the lower the score of RMI, the more likely of mutation of KRAS (Exon 2 Codon 12 and Exon 3 Codon 61) was to be found.

DISCUSSION

Behavior of borderline epithelial neoplasm is between benign and malignant. Complex aberration is not found in borderline tumor and differences between genetic aberration can be seen in borderline tumor and invasive carcinoma, thus implies that high grade invasive tumor occur from existing borderline lesion. Peritoneal implant is found in 60% serous type borderline tumor but might be found in cases without ovarian tumor.[1,3]

Kinase RAS-RAF-MEK-ERK-MAP path is frequently affected in carcinoma; particularly oncogen RAS play pivotal role in tumorigenesis. Mutation of RAS first described in malignant melanoma, pulmonary carcinoma and thyroid papillary. One of 3 proto-oncogen RAS shows mutation in 25% cases of carcinoma; codon 12, 13 and 61 KRAS are mostly affected. Mutation of KRAS shows constitutive activation from protein with the increase exchange of GDP/GTP or decrease of GTPase protein activity, that leads to the increase of cellular proliferation. Mutation of KRAS and BRAF in noninvasive carcinoma and invasive ovarian has been reported previously where mutation of KRAS is commonly found in ovarian tumor of mucinous subtype and in borderline serous tumor, but not in invasive serous carcinoma. Three codes of genes of RAF for kinase cytoplasmic serine/threonine have been regulated with the binding of RAS. Almost 90% of mutation of BRAF occurs upon or soon after activation of exon 15, that protect substrate binding site.[5,8]

In more than 90% cases, mutation of BRAF occur as substitution of adenine (A) with thymidine (T) in the position of nucleotida 1796, that converts valine into glutamic acid in the position of 600 (V600E). Mutation of BRAF leads to activation of ERK, and this promotes transition of G1/S in cellular cycle regulation.[5,8]

In most mutation of BRAF (80-86%) in cancer tranfersion of T>A occur in codon 600 that results in substitution of glutamat to valin. This can increase and change the process of phosphorilation need in the process of activation of BRAF and increase the signal needed in activation of MAPK (mitogen-activated protein kinase), MEK (Mitogen-activated protein kinase kinase) and ERK (extracellular signal-regulated kinase). Some other types of mutations related to codon 600 and the neighboring codon in exon 15 has been identified. Most variations of mutation activate mitogen activated protein kinase kinase (MAPK kinase) that will disturb the signal of activation from extracellular signal-regulated kinase (ERK), but it is rare that the mutation occur in CRAF and ERK that lead to sectioning of path of MAPK kinase.[9,13]

Mutation of BRAF and KRAS is not always found in similar tumor but the type of tumor show mutation of KRAS and BRAF are identical. This supports the hypothesis that mutation of KRAS and BRAF is equivalent for the gumorigenic effect.

This study found mutation of BRAF in 47% of the samples and mutation of KRAS in 94% (serous borderline 62.5%, mucinous borderline 37.5%), with 70.6% mutation of exon 2 codon 12 (serous borderline 33.3%, mucinous borderline 66.7%), 52.9% mutation of exon 2 codon 13 (serous borderline 33.3%, mucinous borderline 66.7%), and 76.6% mutation of exon 3 codon 61 (serous borderline 30.8%, mucinous borderline 69.2%). In the study by Mayr D, it was found that mutation of KRAS or BRAF is commonly found in borderline serous and mucinous tumors. Mutation of BRAF is found in 30% of all borderline serous tumors, but in subjects with invasive mucinous tumor, there was only one case found and in subjects with serous carcinoma, there was no mutation found. Finding of mutation of BRAF in a carcinoma endometrioid type (K600N) and a clear-cell carcinoma (S615F) need further studies at larger scales. Mutation of KRAS was found in almost 50% of mucinous and more than 20% of serous type of borderline tumor but is rare in any invasive carcinoma subtype. The correlation between mutation of BRAF and KRAS in similar cancer type shows activation of RAS-RAF-MAPK-MEK-ERK kinase path can be achieved by activating different mutation activation.[15]

Borderline ovarian tumor of serous and mucinous, may represent pathological correlation bet-

ween benign and invasive carcinomas, but they seem to be heterogeneous groups. Results of Fisher exact test showed that of the five independent variables, only mutation of BRAF show significant result (p=0.009), while others did not give any significant result. Therefore, it seems examination in BRAF may be more prioritized than examination in KRAS. Results of other Exact Fisher tests showed (Table 2), that there was no significant correlation between mutation of KRAS and BRAF and the pathological type. However, there is a tendency in serous borderline type more mutations of BRAF were found, while in mucinous borderline type more mutation of KRAS was found.

RMI scores as a method to estimate malignancy is a standard action in ovarian tumor. Results of Mann-Whitney test in the study to identify the correlation between the use of RMI score to see the possible malignancy with mutation of BRAF and KRAS show insignificant correlation. However it is found that there was a positive tendency in mutation of BRAF, where the higher the score of RMI, the higher the probability of mutation of BRAF and negative correlation with KRAS, the lower the score of RMI the more probably the mutation of KRAS (Exon 2 Codon 12 and Exon 3 Codon 61).

CONCLUSION

Mutation of KRAS occured in 94% of samples (serous borderline 62.5%, mucinous borderline 37.5%), that is mutation of exon 2 codon 12 of 70.6% (serous borderline 33.3%, mucinous borderline 66.7%), mutation of exon 2 codon 13 of 52.9% (serous borderline 33.3%, mucinous borderline 66.7%), and mutation of exon 3 codon 61 of 76.5% (serous borderline 30.8%, borderline mucinous 69.2%),

Mutation of BRAF occured in 47% of samples, but results of Exact Fisher test showed that mutation of BRAF showed significant results, while other variables did not give significant results.

There is no significant correlation between mutation of KRAS and BRAF and pathological type. However, there is a tendency that in serous borderline type there were more mutations of BRAF, while in mucinous borderline type there were more mutations of KRAS.

There was a positive tendency of mutation of BRAF, where the higher the score of RMI, the higher the probability of mutation of BRAF and negative correlation with KRAS, the lower the score of RMI the higher the probability of mutation of KRAS (Exon 2 Codon 12 and Exon 3 Codon 61).

CONFLICT OF INTEREST

No potential conflict of interest

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