ГОРЯЧИЕ ТОЧКИ МУТАЦИЙ ВО ВНЕКЛЕТОЧНЫХ ДОМЕНАХ MICA/MICB

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Резюме. MICA и MICB – это неклассические молекулы MHC, которые являются индикаторами клеточного стресса. Они выполняют функцию лигандов рецепторов NKG2D NK-клеток, вызывая цитотоксический ответ против поврежденных, инфицированных или трансформированных клеток. Образование растворимых форм MICA/MICB происходит путем расщепления их внеклеточных доменов. Экспрессия молекул MICA/MICB на опухолевых срезах или уровни их растворимых форм в крови могут быть использованы при диагностике онкологических заболеваний. Они могут предсказывать важные клинические параметры онкологических больных, такие как общая и безрецидивная выживаемость. Однако их обширный молекулярный полиморфизм затрудняет разработку моноклональных антител (МКАТ) для использования в диагностике. Ввиду этого диагностическая ценность анализов на основе МКАТ может меняться в зависимости от частоты аллельных вариантов в локальных человеческих популяциях. Мы изучили аминокислотные последовательности экстраклеточных доменов более 280 аллельных вариантов MICA и 50 аллельных вариантов MICB. Кроме того, мы выявили 172 и 58 однонуклеотидных полиморфизмов, расположенных в кодирующих областях соответствующих генов и приводящих к аминокислотным заменам. Наиболее частые аминокислотные замены (> 10%) в экстраклеточных доменах происходят в 11 и 4 сайтах MICA и MICB, соответственно. Мы обнаружили, что частоты однонуклеотидных полиморфизмов в выявленных горячих точках выраженно коррелируют друг с другом в различных популяциях человека, несмотря на разнообразие частот аллельных вариантов. Известна функциональная роль только одного сайта. Замена валина на метионин в положении 152 повышает сродство MICA к связыванию с рецептором NKG2D. Поскольку «горячие точки» распределены по всей последовательности экстраклеточных доменов, они могут играть иную роль, нежели модуляция аффинитета взаимодействия с рецептором NKG2D. Мы рекомендуем, чтобы наборы антигенов для валидации МКАТ к MICA и MICB, отвечали двум критериям. Во-первых, они должны включать аллели как MICA, так и MICB, поскольку их аминокислотные по-

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следовательности схожи между собой. Во-вторых, аллели должны покрывать вариабельность, наблюдаемую в выявленных «горячих точках».

Ключевые слова: MICA, MICB, полиморфизм, рак, диагностика, моноклональные антитела

MUTATION HOT SPOTS IN MICA/MICB EXTRACELLULAR DOMAINS

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Abstract. MICA and MICB are non-classical MHC molecules that indicate cellular stress. They act as ligands for NKG2D receptors found on NK cells, thereby triggering a cytotoxic response against damaged, infected, or transformed cells. The production of soluble forms of MICA/MICB occurs via the cleavage of their extracellular domains (ECDs). The expression of MICA/MICB molecules in tumor sections or the levels of their soluble forms in blood have potential as diagnostic tools for cancer. They can predict important clinical outcomes for cancer patients, such as overall and recurrence-free survival. However, their extensive molecular polymorphism complicates the development of monoclonal antibodies (mAbs) for diagnostic use. Therefore, the diagnostic value of mAb-based assays may vary depending on the frequencies of allelic variants in local human populations. We examined the ECD amino acid sequences of more than 280 MICA and 50 MICB allelic variants. Additionally, we identified 172 and 58 single nucleotide polymorphisms (SNPs) located in the coding regions of the respective genes and resulting in amino acid replacements. The most frequent amino acid replacements (> 10%) in the ECD occur at 11 and 4 sites of MICA and MICB, respectively. We found that the frequencies of SNPs in the identified hot spots strongly correlate with each other in different human populations, despite the diversity of allelic variant frequencies. The functional role of only one site is known. The replacement of valine with methionine at position 152 enhances the affinity of MICA to NKG2D receptor. As the hot spots are dispersed throughout the entire ECD sequences, they may play a role other than modulating affinity with the NKG2D receptor interaction. We recommend that Ag sets used to validate anti-MICA/MICB mAbs meet two criteria. First, they should include both MICA and MICB alleles, as these genes have highly similar sequences. Second, the alleles should cover the variability observed in the identified hot spots.

Keywords: MICA, MICB, polymorphism, cancer, diagnostics, monoclonal antibodies

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Introduction

MICA and MICB are non-classical MHC molecules found on the cell membranes of stressed, virus-infected, or transformed cells [2]. Their extracellular domains (ECDs) consist of three immunoglobulin domains. Unlike classical MHC molecules, MICA/MICB do not associate with β2-microglobulin and do not present antigen (Ag) peptides. NK cells recognize their expression via NKG2D receptors and eliminate damaged or dangerous cells. Two domains (α1 and α2) are involved in the interaction with NKG2D receptors. Soluble forms of MICA/MICB are produced as a result of ECD cleavage in the α3 domain. Sequence homology of MICA/MICB molecules is high and reaches 84%.

Similar to MHC molecules, MICA/MICB demonstrate an extensive molecular polymorphism [5]. As of 2023, the IMGT database comprises over 500 sequences that encode in excess of 290

MICA proteins, and more than 200 sequences that encode more than 50 MICB proteins. Human populations significantly differ from each other in frequencies of MICA/MICB allelic variants [2]. The variability of MICA molecules is found in all three functional parts: ECD, transmembrane region, and cytoplasmic tail. The variability in the MICA transmembrane region is well studied and occurs due to short tandem repeats in exon V. Variants A4, A5, A6, A7, A9, and A10 possess a corresponding number of GCT triplets encoding alanine resides. MICA-A9 is more frequent in patients with psoriasis [4], nasopharyngeal carcinoma [12], gastric adenocarcinoma [7], acute lymphoblastic and myeloid leukemias [1]. Variants having an inserted G after five GCT repeats (A5.1 variants) form a premature stop-codon and possess short cytoplasmic tails. Patients having these MICA alleles are more likely to develop pancreatic cancer [9], oral cancer [11], breast cancer [6], and atypical forms of celiac disease [8]. Synonymous or nonsynonymous single nucleotide polymorphisms (SNPs) are found in exons encoding ECD. The role of variability in ECD is less understood. The replacement of valine with methionine at position 152 (or 129 if counting starts from E24) increases the affinity of MICA and NKG2D receptor binding [10]. Patients with the Val/Val phenotype at this position have a significantly higher frequency of multiple myeloma relapse [14] and are predisposed to nasopharyngeal cancer [3]. MICB molecular variability is also due to SNPs, but the functional consequences are yet to be established.

The expression of MICA/MICB molecules in tumor sections, or levels of their soluble forms in blood, could be used for cancer diagnosis. These parameters have been shown to predict important clinical outcomes for cancer patients, including overall and recurrence-free survival [13]. However, the polymorphic nature of MICA/MICB ECDs may significantly affect the way monoclonal antibodies (mAbs) used in diagnostic assays bind to these Ags. Therefore, the diagnostic value of these assays may vary depending on the frequencies of allelic variants in local human populations. We suggest that mAbs used in immunohistochemistry and sandwich ELISA should be validated using sets of allelic MICA/MICB variants. By analyzing publicly available sequence and SNP data, we found that the most frequent amino acid replacements in the ECDs are located in 11 and 4 sites of MICA and MICB, respectively.

Materials and methods

The genetic sequences of MICA/MICB allelic variants for this study were retrieved from the IMGT website (https://imgt.org/). We numbered amino acids starting from the first methionine residue in the leader peptide. ECDs were defined as amino acid sequences from E24 to S297. Multiple alignment of the amino acid sequences was performed using the ClustalW algorithm. Information regarding SNPs located in MICA/MICB genes and their frequencies ("1000 Genomes" project) was obtained from the NCBI website (https://www.ncbi.nlm.nih.gov/snp/). Data analysis was carried out using custom R scripts.

Results and discussion

We carried out multiple alignments of 280 and 50 amino acid sequences of MICA and MICB ECD allelic variants, respectively, excluding those with premature stop codons or partial sequences. We identified 63 MICA and 16 MICB alleles with identical sequences in their extracellular parts. Most MICA variants exhibited differences in amino acid sequence at 11 sites, whereas MICB alleles differed at 4 positions (Table 1).

To investigate the frequency of MICA/MICB mutations in human populations, we identified 172 and 58 SNPs located in the coding regions of the respective genes resulting in amino acid replacements. To our surprise, mutations with high frequencies (> 10%) in the global population were located in the previously defined positions (Table 1). Interestingly, we found a high correlation in the frequencies of identified SNPs among different local human populations (Table 2).

We recommend that Ags sets used to validate anti-MICA and anti-MICB mAbs meet two criteria. First, they should include both MICA and MICB alleles as these genes have highly similar sequences. Second, the alleles should cover the variability observed in the identified hot spots. It may also be useful to include variants with replacements in positions that have lower frequency rates (< 10%). mAbs, or combinations thereof, that cover this variability and exhibit high specificity to either MICA or MICB could be used for diagnostic purposes.

TABLE 1. SNPs IN MICA AND MICB GENES LEADING TO THE MOST FREQUENT AMINO ACID REPLACEMENTS. SEQUENCES OF THE MOST COMMON ALLELES (MICA*008 AND MICB*005) WERE USED AS REFERENCES

Ag	Position	Amino acid variation	Frequencies				
			Europe	East Asia	South Asia	Africa	America
MICA	37	W > G	0.193	0.179	0.237	0.38	0.339
	59	Y > C	0.314	0.301	0.299	0.479	0.408
	145	L > V	0.195	0.122	0.273	0.272	0.212
	152	V > M	0.314	0.301	0.299	0.479	0.408
	196	E > K	0.314	0.300	0.300	0.480	0.405
	198	G > S	0.282	0.422	0.41	0.285	0.350
	229	S > G	0.314	0.300	0.299	0.477	0.406
	233	R > W	0.314	0.300	0.299	0.477	0.406
	236	I > T	0.509	0.422	0.572	0.75	0.618
	238	T > S	0.314	0.300	0.299	0.477	0.406
	274	R > Q	0.509	0.423	0.572	0.752	0.62
MICB	75	D > N	0.232	0.094	0.122	0.154	0.135
	80	K > E	0.263	0.275	0.241	0.383	0.19
	121	I > M	0.09	0.068	0.039	0.119	0.045
	136	D > N	0.262	0.274	0.241	0.383	0.19
N:			1006	1008	978	1322	694

TABLE 2. CORRELATION MATRIX OF FREQUENCIES OF SNPs IN IDENTIFIED POSITIONS ACROSS DIFFERENT HUMAN POPULATIONS

	MICA					MICB				
	Europe	East Asia	South Asia	Africa	America	Europe	East Asia	South Asia	Africa	America
Europe	1	0.811	0.899	0.941	0.967	1	0.774	0.909	0.778	0.977
East Asia		1	0.8	0.623	0.789		1	0.967	1	0.891
South Asia			1	0.758	0.837			1	0.969	0.977
Africa				1	0.966				1	0.893
America					1					1

We found that the frequencies of SNPs in the identified hot spots strongly correlate with each other in different human populations, despite the diversity of allelic variant frequencies. This may indicate that selective pressure maintains SNP frequencies at similar levels in different populations. As mentioned earlier, the functional role of only one replacement is known. The functions of all others are yet to be established. Because the hot spots are scattered throughout the entire ECD sequences, they may play a role other than modulating affinity with the NKG2D receptor interaction.

Conclusion

We conclude that the polymorphic nature of MICA and MICB molecules should be taken into account during the development of mAb-based immunoassays. Antigen sets covering the variability in amino acid replacement hotspots could be used for their validation. Revealing the functional role of these replacements may shed light on the pathogenic mechanisms leading to disease development associated with certain MICA/MICB alleles.

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