

## ORIGINAL ARTICLE

# Protective effect of rs712 polymorphism in a let-7 microRNA-binding of KRAS gene in breast cancer of a Mexican population

Martha Patricia Gallegos-Arreola<sup>1</sup>, Carlos Jovany Briseño-Zuno<sup>1,2</sup>, Luis Eduardo Figuera<sup>1</sup>, Guillermo Moisés Zúñiga-González<sup>3</sup>, Carlos Iván Perales-Mederos<sup>1</sup>, Ana María Puebla-Pérez<sup>4</sup>, Mónica Alejandra Rosales-Reynoso<sup>3</sup>

<sup>1</sup>Genetics Division, Western Biomedical Research Center, Mexican Institute of Social Security, Guadalajara, Jalisco, México;

<sup>2</sup>Human Genetic Doctorate, University Center of Health Sciences, University of Guadalajara, Guadalajara, Jalisco, México;

<sup>3</sup>Molecular Medicine Division, Western Biomedical Research Center, Mexican Institute of Social Security, Guadalajara, Jalisco, México; <sup>4</sup>Inmmunopharmacology Laboratory, Exact and Engineering Sciences University Center, University of Guadalajara, Guadalajara, Jalisco, México.

## Summary

**Purpose:** The rs712 polymorphism in a let-7 microRNA-binding KRAS gene has been associated with different types of cancer, however these associations have been inconsistent. The purpose of this study was to determine the association between rs712 polymorphism in a let-7 microRNA-binding KRAS gene comparing breast cancer (BC) patients with healthy subjects from Mexican population.

**Methods:** The genotyping of the rs712 polymorphism was performed by polymerase chain reaction (PCR) in 437 BC patients and 414 healthy women.

**Results:** The observed frequencies of the rs712 polymorphism indicated an associated protective factor for BC in the dominant GT+TT model [odds ratio (OR) 0.70, 95% confidence interval (CI) 0.51-0.97,  $p=0.040$ ]. An association between genotype and BC patients was evident in chemotherapy re-

sponse (allele GT, OR 0.032, 95% CI 0.002-0.505,  $p=0.014$ ), partial chemotherapy response (genotype GT, OR 0.023, 95% CI 0.001-0.419,  $p=0.011$ ), and gastric and hematological toxicity (genotype GT, OR 0.115, 95% CI 0.028-0.473,  $p=0.003$ ), Luminal A BC patients with gastric and hematological toxicity (genotype TT, OR 0.236, 95% CI 0.069-0.805,  $p=0.021$ ) and tobacco consumption (genotype TT, OR 0.283, 95% CI 0.001-0.802,  $p=0.037$ ) and Luminal B with metastatic lymph node (genotype GT, OR 0.241, 95% CI 0.093-0.626,  $p=0.003$ ).

**Conclusion:** Polymorphism rs712 in KRAS gene was protective factor associated with susceptibility for BC in this sample from Mexican population.

**Key words:** rs712, let-7 KRAS, polymorphism, breast cancer, Mexican population, luminal A, luminal B

## Introduction

Among the gynecological type cancers, breast cancer (BC) is the most frequent type of cancer in women world-over [1]. Its incidence varies between different types of ethnic groups [1]. In Mexico, BC is considered one of the major causes of cancer mortality [1-3]. A study estimated that in México there will be an increase in the incidence of BC by 2030 and that about 38% of the deaths will be in

women older than 60 years [4]. BC is also considered to develop through a gradual accumulation of genetic and epigenetic changes that transform normal breast cells into malignant tumor cells [1,5]. Relevant studies have associated the relationship of KRAS gene with BC [6-10]. KRAS is a protein (first identified in kirsten rat sarcoma virus) participating in the RAS/MAPK intracellular signaling

Corresponding author: Martha Patricia Gallegos Arreola, PhD. Genetics Division, Western Biomedical Research Center, Mexican Institute of Social Security. Sierra Mojada 800, Col. Independencia, Guadalajara, Jalisco 44340, México.  
Tel: +52 33 36170060 (31936), Fax: +52 33 36181756, Email: marthapatriciagallegos08@gmail.com  
Received: 29/03/2019; Accepted: 17/05/2019

pathway. *KRAS* has GTPase action and transmits signals of membrane cell to the nucleus. These signals have important functions in growth, proliferation and cell differentiation [11,12]. The *KRAS* oncogene is part of the *RAS* gene family, which plays a fundamental role in tumorigenesis [12]. The *KRAS* gene in humans is located in 12p12.1 chromosome, contains 6 exons and the 4th exon by means of alternative splicing to origin 2 mRNA transcripts, known as active isoforms (4A) and inactive isoform (4B), while exons 1,5 and 6 are non-coding [12,13]. The proto-oncogene *KRAS* is regulated by proteins and microRNAs molecules that bind to the promoter regions of the gene. MicroRNAs such as let-7, lin-4 and bentam, regulate the cell proliferation and differentiation. They also destabilize the mRNA, repress protein synthesis and have been seen altered in cancer. These are divided into oncomirs (oncogenes) and anti-oncomirs (tumor suppressors). The let-7 microRNA is an oncogene-antioncomir, regulates the levels of *KRAS* protein and decreases the rate of cell proliferation [11-13]. The rs712 polymorphism is the product of one base change of G to T in the antisense chain or A to C in sense chain, located in the let-7 complementary binding sites in 3'-UTR of *KRAS* mRNA. Its function is unknown but it has been hypothesized that altered let-7 binding site affects its function and consequently promote cell proliferation and migration in the breast cell, contributing to BC carcinogenesis [12,14]. The T allele (rs712) showed a frequency in controls of 14-30% among African population, 25% (12-37%) in Mexican population, 50% in European and American populations, and 70% in Indian and United Kingdom populations [15]. Also, the rs712 polymorphism has demonstrated a significant association between different types cancers in some studies [16]. However, in Mexican population, the

association of rs712 polymorphism in BC remains unknown. Thus, the purpose of this investigation was to determine the frequency and association of the rs712 polymorphism in a let-7 microRNA-binding *KRAS* gene in Mexican women with BC.

## Methods

Blood samples were collected from 414 healthy blood donating women and 437 patients with clinically and histologically confirmed BC. The patient and control groups were not age-matched and no family samples were included. All the samples from the study group were recruited from June 2014 to February 2019, and were obtained after the patients and controls provided written informed consent, as approved by the local ethical committee (1305). All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration. Clinical and demographic data were obtained using written questionnaires. All patients were also interviewed to determine occupational exposure and current drug regimens. The BC patient database and patient DNA samples were examined for other polymorphisms and no family samples were included [5].

DNA was extracted from peripheral blood lymphocytes using standard protocols [17].

The PCR amplification of the rs712 polymorphism was performed by primer: 5'-ATGACAGTGGAA-GTTTTTTTTTCCTC-3' and 5'-GAATCATCATCAGGAAGC-CAT-3' [12,18]. The PCR amplifications were performed in a total volume of 15  $\mu$ L with the following PCR conditions: 94°C (4 min), followed by 35 cycles at 94°C (45 sec), 56.5°C (45 sec) and 72°C (50 sec), with a final extension at 72°C (7 min). The PCR product was digested 4 h at 65°C with Taq I restriction enzyme (New England BioLabs Inc; Beverly, MA, USA). The genotypes were identified in 6% polyacrylamide gels (29:1), followed by silver nitrate staining [19]. The GG genotype was determined [wild type, 299 base pairs (bp) and 25 bp] as the digested fragment and TT genotype (polymorphic type, 324bp) as the undigested fragment.

**Table 1.** Demographic data for the study group

| Demographic data    | BC patients (n=437)<br>n (%) | Controls (n=414)<br>n (%) | p value |
|---------------------|------------------------------|---------------------------|---------|
| Age (years)         |                              |                           | 0.004   |
| Mean $\pm$ SD*      | 52.38                        | 50.02                     | 0.189   |
| <50                 | 180 (41)                     | 189 (46)                  |         |
| $\geq$ 50           | 257 (59)                     | 225(54)                   |         |
| Tobacco consumption |                              |                           | <0.0001 |
| No                  | 313(72)                      | 368 (89)                  |         |
| Yes                 | 124(28)                      | 46 (11)                   |         |
| Alcohol consumption |                              |                           | <0.0001 |
| No                  | 330 (76)                     | 372 (90)                  |         |
| Yes                 | 107 (24)                     | 42 (10)                   |         |

SD: standard deviation, \*Student's t-test

Allele frequencies were obtained by direct counting. The Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit  $\chi^2$  test to compare the observed genotype frequencies to the expected frequencies among control subjects. ORs ratios and 95% CI were also calculated. A two-tailed  $p < 0.05$  was considered statistically significant. The association analysis was determined by ORs ratio and binary logistic regression were performed using the PASW Statistic Base 18 software, 2009 (Chicago, IL, USA).

## Results

Comparative epidemiological data from the BC patients and control individuals are shown in Table 1. The observed average age in BC patients was 52.38 years, ranging from 23 to 84 years, and both the tobacco and alcohol consumption were statistically different in BC patients and controls ( $p < 0.0001$ ).

The TT genotype frequency of the rs712 polymorphism on a let-7 microRNA-binding KRAS gene did not show significant differences between patients and controls (Table 2). However, the dominant model (GT+TT) was observed as protective factor present in 79% (347/437) of the BC patients and 73% (302/414) of the controls (OR 0.70, 95%CI

0.51-0.97,  $p = 0.040$ ). The distribution of genotypes in the controls was consistent with Hardy-Weinberg Equilibrium (HWE).

Significant differences were found with regards to clinical characteristics of the BC group and heterozygous (GT) genotype of the rs712 polymorphism. Chemotherapy response (adjusted OR 0.032, 95% CI 0.002-0.505,  $p = 0.014$ ), partial chemotherapy response (adjusted OR 0.023, 95% CI 0.001-0.419,  $p = 0.011$ ), and gastric and hematological toxicity (adjusted OR 0.115, 95% CI 0.028-0.473,  $p = 0.003$ ) were protective factors for susceptibility of BC (Table 3).

In addition, it was observed that rs712 polymorphism served as a protective factor for susceptibility of BC patients stratified by molecular classification (Table 4). Luminal A BC patients [defined by estrogen receptor (ER) positive, human epidermal growth factor receptor 2 (HER2) negative, tumor marker proliferative cell (Ki-67) low and progesterone receptor (PR) high] with TT genotype showed gastric and hematological toxicity during chemotherapy (adjusted OR 0.236, 95% CI 0.069-0.805,  $p = 0.021$ ), and tobacco consumption (adjusted OR 0.283, 95% CI 0.001-0.802,  $p = 0.037$ ). BC patients with luminal B type (defined by ER positive, HER2

**Table 2.** Genotype and allelic distribution of the rs712 polymorphism of KRAS in BC patients and controls

| rs712 polymorphism |          | BC (n=437)   | Controls* (n=414) | OR   | CI (95%)    | p value |
|--------------------|----------|--------------|-------------------|------|-------------|---------|
| Model              | Genotype | n (%)        | n (%)             |      |             |         |
|                    | GG       | 90 (21)      | 112 (27)          | 1    |             |         |
|                    | GT       | 237 (54)     | 208 (50)          | 1.17 | (0.89-1.53) | 0.2727  |
|                    | TT       | 110 (25)     | 94 (23)           | 1.14 | (0.83-1.57) | 0.4460  |
| Dominant           | GG       | 90 (21)      | 112 (27)          |      |             |         |
|                    | GT+TT    | 347 (79)     | 302 (73)          | 0.70 | (0.51-0.97) | 0.040   |
| Recessive          | TT       | 110 (17)     | 94 (25)           |      |             |         |
|                    | GG+GT    | 327 (83)     | 320 (75)          | 1.14 | (0.83-1.57) | 0.4460  |
| Allele (2n=874)    |          | 2n=828       |                   |      |             |         |
|                    | G        | 417 (0.4771) | 432 (0.5217)      | 0.83 | (0.69-1.01) | 0.0731  |
|                    | T        | 457 (0.5229) | 396 (0.4782)      | 1.19 | (0.98-1.44) | 0.0731  |

\* Controls genotype. Hardy-Weinberg equilibrium in controls ( $\chi^2$  test = 0.0187;  $p = 0.8910$ ). OR: odds ratio, CI: confidence interval

**Table 3.** Association between of the rs712 polymorphism of KRAS gene and clinical variable in BC patients

| Genotype | Clinical variables*                  | OR    | 95%CI         | p value |
|----------|--------------------------------------|-------|---------------|---------|
| GT       | **Chemotherapy response              | 0.032 | (0.002-0.505) | 0.014   |
|          | ** Partial chemotherapy response     | 0.023 | (0.001-0.419) | 0.011   |
|          | **gastric and hematological toxicity | 0.115 | (0.028-0.473) | 0.003   |

\*Non-significance clinical variables included in the analysis: age (<50,  $\geq 50$  years), tobacco and alcohol consumption, stage (I-II, III-IV), menopause, body mass index (overweigh, obesity I, II, and III), lymph node metastasis, Luminal A, Luminal B, HER2, Ki67, non-chemotherapy response, non-chemotherapy response by recurrence. OR: odds ratio, CI: confidence intervals. \*\*The non-response to chemotherapy with anthracyclines (e.g. doxorubicin, epirubicin, liposomal doxorubicin), taxanes (docetaxel, paclitaxel) and trastuzumab was evaluated according to the pathological Ryan's classification described as follows: 1. Moderate response (single cells or small groups of cancer cells), 2. Minimum response (residual cancer surrounded by fibrosis), and 3. Poor response (minimal or no tumor destruction, extensive residual cancer) [26].

**Table 4.** Association between the rs712 polymorphism of KRAS gene and clinical variables stratified by histological status in BC patients

| Genotype | Histology | Clinical variables*                  | OR    | 95%CI         | p value |
|----------|-----------|--------------------------------------|-------|---------------|---------|
| TT       | Luminal A | **Gastric and hematological toxicity | 0.236 | (0.069-0.805) | 0.021   |
|          |           | **Tobacco consumption                | 0.283 | (0.001-0.802) | 0.037   |
| GT       | Luminal B | **lymph node metastasis              | 0.241 | (0.093-0.626) | 0.003   |

For details see footnote of Table 3

negative and Ki-67 high and/or PR low) with *GT* genotype and lymph nodes positive (adjusted OR 0.241, 95% CI 0.093-0.626,  $p=0.003$ ) was observed as protective genotype.

## Discussion

In Mexico, BC is the principal cause of cancer-related mortality in women [1-5]. In the present study it was observed that 59% of BC patients were  $\geq 50$  years old. Many studies observed a high incidence of BC in patients who were approximately 50 years old [1-5]. The tobacco and alcohol consumption were found to be risk factors and these associations were consistent with those observed previously in multiple studies [20,21]. Lifestyle changes and changes in longevity may have influenced in the increased frequency of BC in Mexican population [1,2,5].

Many relevant studies of BC have been associated with different polymorphisms on *KRAS* gene, especially in those that present DIANA regions to proteins and microRNAs such as rs712 polymorphism that had described to alter the let-7 binding site that regulates *KRAS* activity, affecting gene expression and promoting the proliferation in the BC cells [12,22,23].

Studies of rs712 polymorphism have demonstrated an association with a risk susceptibility to various cancer types including lung [24], gastric [25], colorectal [12,23,26,27], liver [28]. On the contrary, in BC its association has been observed as a protective factor of susceptibility [6,7]. Moreover, little is known regarding to association of rs712 polymorphism in Mexican BC patients. In our study group, the frequency of *TT* variant was not significantly statistically different between BC patients (25%) and controls (23%). However, the dominant model indicates that this polymorphism was a protective factor for susceptibility of BC. These data are in accordance with a recent study that demonstrated the association with decreased risk of the *T* allele, and recessive model of rs712 polymorphism in BC in Iranian population [6]. Another study demonstrated that let-7 rs712 *GT* geno-

type was shown as protective factor in BC tumor metastasis of Chinese population [7]. In retrospect, previous studies have shown that let-7 microRNA might regulate self-renewal and tumorigenicity in BC and decrease potential tumor metastasis [12,29].

Nevertheless, *GT* genotype was seen to be protective factor to toxicity in BC patients with complete and partial response to chemotherapy; gastric (GI: nausea, diarrhea, vomiting, stomatitis, mucositis, and hematological: neutropenia, anemia, and thrombocytopenia). A variety of mechanisms for let-7 activity have been proposed that could contribute to regulation of the expression of distinct pathways required in the tumor behavior [30]. In addition to these mechanisms, several factors could influence chemotherapy, such as the response and the drug toxicity. It has been seen that it is not a mono-factorial event, and also depends on genes encoding proteins involved in multiple metabolic pathways, posttranslational modifications, gene interactions, and epigenetics [12,31]. Thus, this polymorphism located in regulator sequences as microRNAs has been associated in multiple diseases including BC risk in certain populations. These polymorphisms are in the mature microRNA sequence and in the pre- and pri- forms of the microRNA, that can alter their binding sites in target genes and the processing of the protein [30]. Mulrane et al observed the association of polymorphisms in microRNAs sequence with decreased risk of BC [30]. Dysregulation of microRNAs affects drug metabolism with consequences in the toxicity and chemotherapy response. In one BC study oncogenic microRNAs have been associated with resistance to radiotherapy and chemotherapy [30].

In retrospect, it has been observed that let-7 inhibits IL-6 signaling pathway producing a positive feedback loop, via activation of NF- $\kappa$ B, and promoting an important role in the sensitivity to chemotherapy for cancer [32]. Other mechanisms proposed that overexpression of let-7a or decreasing of RNA-binding protein LIN28A expression, turn cancer cells more sensitive to chemotherapy or radiation [33]. It has also been observed that in colorectal cancer patients with *KRAS* wild-type

metastatic and high let-7c had better response to EGFR monoclonal antibodies [34]. These studies suggested that let-7 has an important function in the regulation of drug metabolism [32].

rs712 polymorphism exhibited a protective effect in BC patients with gastric and hematological toxicity under chemotherapy in this study. It could be thought that the rs712 allows chemotherapeutic drug to remain in the cell for a longer period, thus producing a more efficient elimination of tumor cells. This could cause greater toxicity in the cell, allowing adverse effects of gastric and hematological toxicity. More studies are necessary to know about the multiple functions of let-7.

Studies of other polymorphisms of microRNAs have reported significant associations with reduce risk of T allele of MIR196A2 polymorphism in different cancer types including BC from Caucasian and Asian populations. These results strongly suggest that reduced risk associated with MIR196A2 variant genotype is not mediated by differential expression of the gene's mature miRNAs [35].

In this study we also observed protective activity of the association between TT genotype in BC Luminal A with gastric and hematological toxicity during chemotherapy and tobacco consumption, as well as reduced risk of GT genotype in BC luminal B with lymph node metastasis. Investigations on the expression of microRNAs showed different molecular subtypes of BC, including Luminal A, Luminal B expression profiles of the different microRNAs. These depend on the time of therapy, before the operation or after chemotherapy and radiotherapy, which could be predictive factors for prognosis of patients with BC. The findings suggested that the microRNAs can be used to identify the different nature of breast tissues, and deregulation of the selected microRNAs may affect critical molecular events involved in tumor progression [36,37].

## References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68:394-24.
2. Hunt BR. Breast Cancer Prevalence and Mortality among Hispanic Subgroups in the United States, 2009-2013. *J Cancer Epidemiol* 2016, ID 8784040. doi: 10.1155/2016/8784040.
3. Soto E, Chavarri Y. National and regional breast cancer incidence and mortality trends in Mexico 2001-2011: Analysis of a population-based database. *Cancer Epidemiol* 2016;41:24-33.
4. Mohar A, Reynoso N, Armas D, Gutiérrez C, Torres JA. Cancer Trends in Mexico: Essential Data for the Creation and Follow-Up of Public Policies. *J Glob Oncol* 2017;3:740-8.
5. Gallegos MP, Márquez MG, Sánchez-Corona J et al. Association of the Del1518 Promoter (rs3730485) Polymorphism in the MDM2 Gene with Breast Cancer in a Mexican Population. *Ann Clin Lab Sci* 2017;47:291-7.
6. Sanaei S, Hashemi M, Eskandari E, Hashemi SM, Bahari G. KRAS Gene Polymorphisms and their Impact on Breast Cancer Risk in an Iranian Population. *Asian Pac J Cancer Prev* 2017;18:1301-5.

With respect to the association between TT genotype in BC Luminal A with tobacco consumption as protective factor, several authors reported that different polymorphisms have been associated with the mechanism of BC and tobacco use, yet these associations have been controversial. Also, it has been hypothesized that such effects could be due to a nicotine receptor [24].

On the other hand, we observed a reduced risk of GT genotype in BC luminal B with lymph node metastasis. Similar data was reported in BC patients from China [7].

In conclusion, our results showed an association protective activity for BC compared to controls and BC patients in the dominant model (GT+TT genotype). However, the differences were most evident in patients with GT genotype with: 1) chemotherapy response; 2) partial chemotherapy response; 3) gastric and hematologic toxicity during chemotherapy, as well as in luminal A BC with TT genotype and presence of gastric and hematologic toxic and tobacco consumption. In luminal B BC with GT genotype and lymph node metastasis it was confirmed that these factors significantly contributed to BC susceptibility in the analyzed sample from Mexican population. Further studies are required to confirm these observations.

## Acknowledgements

The authors would like to thank the nurses for assisting in the extracting blood samples. This research was financially supported through FIS/IMSS/PROT/ Application Number 2019-3-60.

## Conflict of interests

The authors declare no conflict of interests.

7. Huang X, Yang Y, Guo Y et al. Association of a let-7 KRAS rs712 polymorphism with the risk of breast cancer. *Genet Mol Res* 2015;14:16913-20.
8. Uvirova M, Simova J, Kubova B et al. Comparison of the prevalence of KRAS-LCS6 polymorphism (rs61764370) within different tumour types (colorectal, breast, non-small cell lung cancer and brain tumours). A study of the Czech population. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2015;159:466-71.
9. Cerne JZ, Stegel V, Gersak K, Novakovic S. KRAS rs61764370 is associated with HER2-overexpressed and poorly-differentiated breast cancer in hormone replacement therapy users: a case control study. *BMC Cancer* 2012;12:105.
10. Paranjape T, Heneghan H, Lindner R et al. A 3'-untranslated region KRAS variant and triple-negative breast cancer: a case-control and genetic analysis. *Lancet Oncol* 2011;12:377-86.
11. OMIM \*190070. V-KI-RAS2 Kirsten rat sarcoma viral oncogene homolog; KRAS. <https://www.omim.org/entry/190070>.
12. Gallegos MP, Zúñiga GM, Gómez K et al. Association of rs712 polymorphism in a let-7 microRNA-binding site of KRAS gene with colorectal cancer in a Mexican population. *Iran J Basic Med Sci* 2019;22:323-26.
13. Jancík S, Drábek J, Radzioch D, Hajdúch M. Clinical relevance of KRAS in human cancers. *J Biomed Biotechnol* 2010;150960. doi: 10.1155/2010/150960
14. Jiang QH, Peng HX, Zhang Y, Tian P, Xi ZL, Chen H. rs712 polymorphism within let-7 microRNA-binding site might be involved in the initiation and progression of colorectal cancer in Chinese population. *Oncotargets Ther* 2015;8:3041-45.
15. U.S. National Library of Medicine. dbSNP short genetic variation. Rs712 <https://www.ncbi.nlm.nih.gov/snp/rs712>
16. Du XY, Hu YY, Xie C et al. Significant association between Let-7-KRAS rs712 G > T polymorphism and cancer risk in the Chinese population: a meta-analysis. *Oncotarget* 2017;8:13863-71.
17. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
18. Li ZH, Pan XM, Han BW et al. A let-7 binding site polymorphism rs712 in the KRAS 3' UTR is associated with an increased risk of gastric cancer. *Tumour Biol* 2013;34:3159-63.
19. Sanguinetti CJ, Dias E, Simpson AJ. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 1994;17:914-21.
20. Bea VJ, Cunningham JE, Alberg AJ et al. Alcohol and Tobacco Use in an Ethnically Diverse Sample of Breast Cancer Patients, Including Sea Island African Americans: Implications for Survivorship. *Front Oncol* 2018;8:392.
21. Gallegos MP, Figuera LE, Ramos A. The association between the 844ins68 polymorphism in the CBS gene and breast cancer. *Arch Med Sci* 2014; 10:1214-24.
22. Chan SH, Wang LH. Regulation of cancer metastasis by microRNAs. *J Biomed Sci* 2015; 22:9. doi: 10.1186/s12929-015-0113-7.
23. Zhang W, Winder T, Ning Y et al. A let-7 microRNA-binding site polymorphism in 3'-untranslated regions of KRAS gene predicts response in wild-type KRAS patients with metastatic colorectal cancer treated with cetuximab monotherapy. *Ann Oncol* 2011;22:104-9.
24. Chin LJ, Ratner E, Leng S et al. A SNP in a let-7 microRNA Complementary Site in the KRAS 3' Untranslated Region Increases Non-Small Cell Lung Cancer Risk. *Cancer Res* 2008 68;8535-40.
25. Li ZH, Pan XM, Han BW et al. A let-7 binding site polymorphism rs712 in the KRAS 3' UTR is associated with an increased risk of gastric cancer. *Tumor Biol* 2013;34:3159-63.
26. Pan XM, Sun RF, Li ZH et al. A KRAS rs712 polymorphism increases colorectal cancer risk. *Tumor Biol* 2014;35:831-35.
27. Wei-Hai Z, Xiao-Fei Q, Zhe-Gang X, Li-Qin Z, Long Q, Chao L. Association of rs712 Polymorphism in Kras Gene 3'-untranslated Region and Cancer Risk: A Meta-Analysis. *J BUON* 2015;20:309-16.
28. Xiong D, Song YP, Xiong W, Liang YD. An KRAS rs712 polymorphism increases hepatocellular carcinoma risk. *Genet Molec Res* 2015;14:14050-55.
29. Yu F, Yao H, Zhu P et al. let-7 regulates self renewal and tumorigenicity of breast cancer cells. *Cell* 2007;131:1109-23
30. Mulrane L, McGee SF, Gallagher WM, O'Connor DP. miRNA dysregulation in breast cancer. *Cancer Res* 2013;73:6554-62.
31. Pinedo HM, Peters GF. Fluorouracil: biochemistry and pharmacology. *J Clin Oncol* 1988;6:1653-64.
32. Sugimura K, Miyata H, Tanaka K et al. Let-7 expression is a significant determinant of response to chemotherapy through the regulation of IL-6/STAT3 pathway in esophageal squamous cell carcinoma. *Clin Cancer Res* 2012;18:5144-53.
33. Balzeau J, Menezes MR, Cao S, Hagan JP. The LIN28/let-7 Pathway in Cancer. *Front Genet* 2017;8:31.
34. Mizuno R, Kawada K, Sakai Y. The Molecular Basis and Therapeutic Potential of Let-7 MicroRNAs against Colorectal Cancer. *Can J Gastroenterol Hepatol*. 2018;5769591. doi: 10.1155/2018/5769591.
35. Christensen BC, Avissar-Whiting M, Ouellet LG et al. Mature miRNA sequence polymorphism in MIR196A2 is associated with risk and prognosis of head and neck cancer. *Clin Cancer Res* 2010;16:3713-20.
36. Khalighfar S, Alizadeh AM, Irani S, Omranipour R. Plasma miR-21, miR-155, miR-10b, and Let-7a as the potential biomarkers for the monitoring of breast cancer patients. *Sci Rep* 2018;8:17981.
37. Kurozumi S, Yamaguchi Y, Kurosumi M, Ohira M, Matsumoto H, Horiguchi J. Recent trends in microRNA research into breast cancer with particular focus on the associations between microRNAs and intrinsic subtypes. *J Hum Genet* 2017;62:15-24.