Int J Cancer Manag. 2022 August; 15(8):e118832. doi: 10.5812/ijcm-118832.

Published online 2022 September 18. **Research Article**

The Association of rs5745687 Polymorphism Located at *HGF* Gene with Risk of Gastric and Breast Cancer in the Helicobacter Positive Patients of Isfahan Population

Mehrnoush Azadeh Jouneghani [1], Fatemeh Keshavarzi [1,*], Nahid Haghnazari [1], Sabrieh Amini [1] and Zahra Hooshmandi [1]

[1] Department of Biology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran

[*] *Corresponding author*: Department of Biology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran. Email: gol.keshavarzi@gmail.com

Received 2021 August 28; **Revised** 2022 July 24; **Accepted** 2022 September 10.

Abstract

Background: *Hepatocyte growth factor* (*HGF*) protein regulates cell growth, motility, and morphogenesis in a variety of cells and tissues by binding to the *HGF* receptor. The rs5745687 SNPs in the introns of the *HGF* gene could affect the splicing and expression of *HGF* mRNA.

Objectives: In this study, the genotype frequency of rs5745687 in breast cancer (BC) and gastric cancer (GC) (positive helicobacter) patients has been investigated and compared with the healthy controls in the Isfahan population.

Methods: Firstly, initial bioinformatics studies were done. Then, according to the results, bioinformatics high-resolution melt (HRM) and real-time PCR were recruited to determine genotypes rs5745678 for 432 participants in the case-control analysis (84 GC with 126 healthy control samples, as well as 111 BC cases with 111 normal controls). The conditional logistic regression model was used to measure odds ratios (OR) and 95% confidence intervals (CI) to produce these cancers based on genotype frequency.

Results: The homozygote genotype of the mutant (G) allele of rs5745678 has a significant association with the lower risk of gastric cancer (P-value < 0.0001) and this allele can increase the risk of GC in a co-dominant model (OR: 5.541, P-value < 0.0001). Also, the rs5745678 SNP had a significant association with the clinicopathological features (age, smoking, *Helicobacter Pylori* infection) in GC patients.

Conclusions: The presence of a single G allele in rs5745678 heterozygote (AG/AA) and co-dominant (AG/AA+GG) models could significantly impact GC pathogenicity in different ways. There was no significant correlation between the rs5745678 polymorphism and BC (P-value: 0.671) in the studied sample size.

Keywords: Gastric Cancer, Breast Cancer, *HGF* Gene

1. Background

Despite developments in systemic chemotherapy, the most prominent cause of cancer-related deaths in gastric adenocarcinoma (GC) and breast cancer (BC) occur in poor prognosis. The heterogeneity of these cancers suggests that novel biomarkers should be developed to identify tumors and improve individually tailored treatments (1).

Genome-wide association studies (GWAS) have linked 3800 SNPs to 427 diseases and characteristics, which is 7% of SNPs found in protein-coding regions (2), but 93% in the non-coding areas (1, 3, 4). SNPs' role in cancer has been demonstrated in several studies, among which miRNA-related studies have recently gained greater interest (2, 5-9).

The rs5745678 SNP located at the *Hepatocyte growth factor* (*HGF*) gene can cause illness by influencing promoter activity of the *HGF* (gene expression) gene, *HGF* mRNA structure (stability), and subcellular localization of *HGF* mRNA or protein (10).

Over the last 2 decades, the functional role of c-Met signaling in the *HGF* receptor (c-Met) pathway has been demonstrated by high-quality preclinical and clinical studies (11, 12).

The *HGF* gene on chromosome 7, also known as the scatter factor, has 17 introns and 18 exons (13). First *HGF* synthesizes and secrete as an inactive precursor (pro-*HGF*), then convert into an active 90 KD heterodimer consisting of an alpha chain and a beta chain. *HGF* receptors can be observed in the surface of gastric (14) and breast (15) tissue. Binding *HGF* to c-Met triggers tyrosine kinase (16) residue

phosphorylation within c-Met and leads to carcinogenesis by sequential c-Met signaling pathways.

Clinical studies have verified the importance of *HGF* involvement in cancer growth and progression. Also, the degree of expression of *HGF* and c-Met has been shown to associate with disease progression and poor patient prognosis (17).

This research aimed at assessing the association of various genotypes of rs5745678 polymorphism with the development of GC and BC in patients in comparison with stable control specimens.

2. Objectives

The current study aimed at assessing the association of *HGF* rs5745687 polymorphism with the risk of BC and GC (positive helicobacter) in the Isfahan population.

3. Methods

This research was a case-control study and ethically approved by the Ethics Committee of Sanandaj Branch, Islamic Azad University, Sanandaj, Iran (IR.IAU.SDJ.REC.1401.008). Also, all protocols affecting human subjects are compliant with the requirements of the Declaration of Helsinki of the Iranian Ministry of Health and Medical Education.

3.1. Silico Analysis

Firstly, initial bioinformatics studies were done. The results revealed that specific miRNAs' binding might be impaired by the rs5745678 locus replacement of various alleles. Across the potential miRNAs, hsa-miR-320e seemed to be more relevant as allele G's existence enhanced the binding affinity (ΔG = -18.91 kcal/mol). This indicates that allele G could facilitate the down-regulation of the miR-320e-mediated *HGF*.

3.2. Inclusion Criteria

A two-stage case-control analysis was performed in a joint dataset of 432 people (111 BC cases and 111 controls, 84 GC and 126 healthy control).

Diagnosis of the stage of the disease and histological examinations of BC patients were carried out at AL Zahra Cancer Institute and Specialized Hospital (S). Also, the control groups (genetically unrelated to the patients) were without a personal history of malignant tumors and belonged to the local population of Isfahan, and were age range-matched with the same cases.

The age range of patients (BC & GC) was 25 to 75 years. The number of BC patients in stages 1, 2, 3, and 4 were 21, 25, 12, and 53, respectively, and 81% of them had tumor sizes over 5 cm. Also, the ratio of menopausal and non-menopausal BC patients was almost equal. The number of patients in terms of estrogen, progesterone, and Her2 receptors were for all 3 receptors, respectively: 47 positive, 36 negative and 28 unconfirmed, 58 positive, 15 negative and 38 unconfirmed, 47 positive and 46 negative, and 18 people not approved. In GC patients, the tumor site was from all parts of the stomach. The ratio of smokers to non-smokers was almost equal and all blood types were found in patients. Also, 25 patients were positive *Helicobacter pylori*, 37 were negative, and 22 were not confirmed. In addition, the ratio of metastatic to non-metastatic individuals was almost equal and 42% of patients were in disease stage 2.

3.3. Exclusion Criteria

The exclusion criteria were that none of the patients were undergone radiotherapy and chemotherapy.

3.4. SNP Genotyping

Firstly, 5 mL blood sample was taken from each participant (patient and control). Then, DNA extraction was done, using Kit (Ex gene^{TM} Clinic SV [Gene All C., Korea]) according to the manufacturer's company instructions and the quality of Dna extracts was determined by a NanoDrop ND-1000 Spectrophotometer (Thermos Fisher Scientific, Inc., Waltham, MA). Using unique primers of rs5745678 that were designed by oligo 7 software (Fwd: 5'GTCTTTTAGCAGTCCATACAATC3', Rwd: 5'GAGGAGAGGACCAAGTTCACA3'), PCR was performed in the final volume of 25 μL, using Sinagene PCR kit. According to the brochure, 12.5 μL Mastermix, 8.5 μL ddH2O, 1 μL forward primer, and 1 μL reverse primer were combined and 23 μL were removed from the combined materials and placed in microtubes. Then, 2 μL of DNA were added to each microtube and pipetted slowly. A control sample was also prepared; then, the samples were shrunk, using a microspin machine and placed inside the PCR machine. The thermocycler program included the initial denaturation in 94°C for 4 minutes, 35 cycles of denaturation in 94°C for 45 seconds, the annealing in 58°C for 45 seconds, and extension in 72°C for 45 seconds, and, then, MICPCR software was used to determine the samples' genotype and analyze the diagram based on the HRM technique or high-temperature melting curve analysis.

3.5. Statistical Analysis

A Chi-square test was performed to compare the genotype frequency in control and tumor samples. The different genotype models were estimated by the logistic re-

gression test by computing odd ratio (OR), confidence interval (CI), and P-value. All mentioned statistical analyses were performed by the IBM SPSS software. The significance level of the statistical analyses is 0.05. Pearson's χ^2, Log-likelihood ratio (Llr) χ^2, and exact tests were used to look into the consistency of the Hardy-Weinberg equilibrium. In addition, the χ^2 test was used to analyze the association test.

4. Results

4.1. Statistical Analysis

Generally, 432 samples were genotyped at position rs5745678, including 84 patients with gastric cancer and 126 healthy controls, depending on the patients' pathological status. Table 1 displays the frequency of AA, AG, and GG genotypes located at the rs5745678 position. The Hardy-Weinberg test revealed that the population evaluated in this analysis was in balance, following the equilibrium requirements of Hardy-Weinberg ($P < 0.0001$). On the other hand, as indicated in Table 2, no significant difference was observed between 111 breast cancer patients and 111 control subjects for rs5745678 SNP ($P = 0.671$).

Table 3 displays the frequency of AA, AG, and GG genotypes of rs5745678 loci in GC patients and healthy controls. The prevalence of AA, AG, and GG genotypes were 34 (40.5%), 10 (11.9%), 40 (47.6%) and 87 (69.0%), 3 (2.4%) and 36 (28.6%) in GC patients and controls, respectively. Also, the G allele had a higher percentage in the cancer group (61.9%) than the A allele; so, the A allele had a higher rate in the control group (63.1%) than the G allele.

Table 4 displays the frequency of CC, TC, and TT genotypes of rs5745678 loci in BC and healthy controls. The prevalence of CC, TC, and TT genotypes were 48 (43.2%), 12 (10.8%), 51 (46%) and 87 (69.0%), 15 (13.5%), and 45 (40.5%) in controls and BC patients, respectively. There was no significant difference in the frequency of genotypes in this SNP in the breast cancer samples, compared to the control.

The AA/GG, AG/GG, and even the dominant, recessive, and co-dominant variants were examined; The AA genotype had a higher occurrence in the control group than in the case group; so, it could be further concluded that the AA genotype may have been associated with a reduced association of cancer.

To investigate the effect of different genotypes on gastric cancer occurrence, we examined GG / AA models and Recessive, Dominant, co-dominant, and Allelic models and calculated OR, CI, and P-values for each model. The co-dominant model with the highest OR rate was selected as the signature model (OR = 5.541, P-value = 0.005). This means that the AG genotype as a co-dominant model increases gastric cancer phenotype probability by 5.541 times. The AG genotype's frequency table was shown as a co-dominant model compared to the AA + GG genotype. The frequency of AG in the CASE group was higher than in the control group and the frequency of GG + AA in the normal group was higher than in the tumor group (Table 5).

4.2. Clinicopathological Result

There was a significant association between age and smoking status in various circumstances and *H. Pylori* status with genotype in the sample population. In patients under 45 years of age, the GG genotype prevalence was higher than in patients over 45 years of age with cancer.

The prevalence of the AG genotype in people with cancer was more remarkable in people over 45 years of age than in people under 45. The prevalence of the AA genotype, on the other hand, was higher in people over 45 years of age; besides, compared to non-smokers with gastric cancer, the frequency of the AG genotype was higher in smokers with cancer. On the other hand, the frequency of AA in cancer smokers was more elevated than in non-smokers (Table 1).

For *Helicobacter pylori* status, the frequency of GG genotype in positive cases was less than negative. AG genotype frequency was higher in positive individuals than negative individuals in the tumor group and AA frequency was higher in the negative pylori group in the case group. On the other side, differences in the frequency of genotypes in all 3 different pathological conditions showed a significant relationship with cancer risk (Table 1). There was no significant difference between the genotype frequency of rs5745678 and the different clinicopathological statuses (Age, ER receptor, PR receptor, and HER2 receptor) of the normal and tumor breast cancer patients (Table 2).

5. Discussion

Initial bioinformatics studies revealed that specific miRNAs' binding might be impaired by the rs5745678 locus replacement of various alleles. Across the potential miRNAs, hsa-miR-320e seemed to be more relevant as allele G's existence enhanced the binding affinity (ΔG =-18.91 kcal/mol). This indicates that allele G could facilitate the down-regulation of the miR-320-mediated *HGF*.

The c-Met pathway is an RTK that stimulates several different molecular signaling pathways after binding its ligand with *HGF*. Also, this pathway is involved in the regulation of cell proliferation, invasion, and angiogenesis of cellular products (18). The c-Met pathway, as observed in tumor biopsies in a range of malignancies, is aberrantly activated or overexpressed. C-Met deregulation is highly associated with poor prognosis and metastatic progression.

Table 1. The Relationship Between rs5745678 Genotypes, Smoking, *Helicobacter pylori* Infection, and Age in Gastric Cancer Patients

Clinical Data, Status, Genotypes	Controls, No. (%)	Cases, No. (%)	P-Value
Age			0.000
< 45			
AA	63 (70.0)	13 (37.1)	
AG	3 (3.3)	4 (11.4)	
GG	24 (26.7)	18 (51.4)	
> 45			
AA	24 (66.7)	21 (42.9)	
AG	0 (0.0)	6 (12.2)	
GG	12 (33.3)	22 (44.9)	
Smoking			0.000
No			
AA	31 (70.5)	13 (40.6)	
AG	1 (2.3)	3 (9.4)	
GG	12 (27.3)	16 (50.0)	
Yes			
AA	18 (78.3)	8 (47.1)	
AG	1 (4.3)	3 (17.6)	
GG	4 (17.4)	6 (35.3)	
H. pilory			0.000
No			
AA	12 (92.3)	17 (40.5)	
AG	1 (7.7)	5 (11.9)	
GG	0 (0.0)	20 (47.6)	
Yes			
AA	75 (66.4)	6 (40.0)	
AG	2 (1.8)	2 (13.3)	
GG	36 (31.9)	7 (46.7)	

Therefore, it can typically occur through various molecular functions, including gene expression and stimulation mediated by increased autocrine or paracrine ligand. C-Met overexpression has been correlated with tumor progression, including lung, ovary, breast, kidney, liver, thyroid, colon, and gastric carcinomas in recent studies. More importantly, MET is a necessary oncogene as well as a subordinate gene responsible for the malignancies' metastatic actions. C-Met has been identified as an independent prognostic factor for bad results for all of these cancer types (2, 7, 8, 19, 20). Both these results reinforce the theory that the *HGF*/c-Met pathway is a crucial cancer regulator and offers an interesting explanation for the rigorous study of c-Met targeting in patients with gastric cancer (2, 21).

HGF is a pleiotropic cytokine secreted by mesenchymal cells, which is the c-Met ligand with the greatest affinity. When secreted, it is inactivated and its extracellular heterodimer compartment is activated automatically by a variety of proteases. *HGF* binds and stimulates c-Met on epithelial cells in a paracrine manner afterward (22). SRC homology-2 domain (SH2)-mediated interactions are involved in the activation of signaling pathways (23-26).

Previous studies revealed that *HGF* had a significant association with the survival rate of gastric cancer patients (27-30). However, there was no previous study about the role of different SNPs of *HGF* in the increasing or decreasing of the GC risk. Although, previous studies revealed that the different SNPs on the different regions of *HGF* play a significant role in cancer development. According to the study by Choi et al. in 2014, rs2074724 in the *HGF* gene

Table 2. The Genotype Frequency of rs5745678 in the Different Clinicopathological Statuses of Breast Cancer Patients

Clinical Data, Status, Genotypes	Controls, No. (%)	Cases, No. (%)	P-Value
Age			> 0.05
< 45			
AA	45 (40.54)	42 (37.84)	
AG	9 (8.11)	7 (6.31)	
GG	38 (34.23)	43 (38.74)	
> 45			
AA	9 (8.11)	11 (9.91)	
AG	2 (1.80)	4 (3.60)	
GG	8 (7.21)	4 (3.60)	
ER receptor			> 0.05
Positive			
AA	12 (10.81)	16 (19.28)	
AG	6 (5.41)	6 (7.23)	
GG	13 (11.71)	19 (22.89)	
Negative			
AA	25 (22.52)	28 (33.73)	
AG	13 (11.71)	5 (6.02)	
GG	42 (37.84)	9 (10.84%)	
PR receptor			> 0.05
Positive			
AA	15 (13.51)	13 (22.41)	
AG	11 (9.91)	5 (8.62)	
GG	15 (13.51)	10 (17.24)	
Negative			
AA	22 (19.82)	19 (32.76)	
AG	8 (7.21)	5 (8.62)	
GG	38 (34.23)	6 (10.34)	
HER2/neu receptor			> 0.05
Positive			
AA	23 (20.72)	17 (18.28)	
AG	20 (18.02)	2 (2.15)	
GG	25 (22.52)	17 (18.28)	
Negative			
AA	14 (12.61)	26 (27.96)	
AG	10 (9.01)	15 (16.13)	
GG	19 (17.12)	16 (17.20)	

is a significant SNP in the breast cancer disease-free survival rate (31). Sui73 and H28 cells both have the single-nucleotide polymorphism (SNP) rs72525097 in intron 1. Together, it was discovered that poly (dA) defects in the *HGF* promoter were prevalent in several cancers, including mesothelioma, colorectal, pancreatic, and lung cancer (32). Carriers of the rare allele (T-allele) of SNP rs975263 had a worse prognosis for developing cancer (HR = 2.17; P

Table 3. Frequency of Genotypes and Alleles in GC and Control Groups

rs5745678	Control, No. (%)	GC, No. (%)	P-Value
Subject	126 (100)	84 (100)	
Genotype			< 0.0001
AA	87 (69.0)	34 (40.5)	
AG	3 (2.4)	10 (11.9)	
GG	36 (28.6)	40 (47.6)	
Allele			0.805
A	159 (63.1)	104 (61.9)	
G	93 (36.9)	64 (38.1)	

Table 4. The Genotype Frequency of rs5745678 in the BC Samples

rs5745678	Control, No. (%)	GC, No. (%)	P-Value
Subject	111 (100)	111 (100)	
Genotype			0.671
CC	48 (43.2)	51 (46)	
TC	12 (10.8)	15 (13.5)	
TT	51 (46)	45 (40.5)	
Allele			0.115
C	108 (48.65)	117 (52.07)	
T	114 (51.35)	105 (47.29)	

Table 5. Association Between Allele Frequency and Gastric Cancer Risk in 6 Different Models

Models	OR	CI (95% Confidence Interval)		P-Value
		Lower	Upper	
GG/AA	2.843	1.560	5.180	0.001
AG/AA	8.529	2.212	32.893	< 0.0001
GG/AG+AA [a]	2.273	1.277	4.046	0.005
AG+GG/AA [b]	3.281	1.843	5.839	0.000
AG/AA+GG [c]	5.541	1.477	20.783	0.005
G/A [d]	0.950	0.635	1.422	0.805

[a] Recessive.
[b] Dominant.
[c] Codominant.
[d] Allelic.

= 0.007 and HR = 2.80; P = 0.003 for event-free survival and overall survival, respectively). Both event-free survival and overall survival were significantly improved by the rare allele (C-allele) of SNP rs3735615 (HR = 0.25; P = 0.001 and HR = 0.16; P = 0.001, respectively) (33).

In this study, for the first time, we demonstrated that there was a significant association between the genotype frequency of rs5745678 in the *HGF* gene and the risk of GC. There was no previous study about the possible role of rs5745678 in the risk of GC. However, it is highly recommended that the expression pattern of *HGF* and the other relevant factors such as has-miR-320e be performed on a larger amount sample in breast and gastric cancers, which could represent valuable information about the effect of this mRNA and other biological factors on breast and gastric cancers.

All in all, we represented that the frequency of G allele and GG genotype in the rs5745678 region of *HGF* has a significant increase in gastric cancer patients, as compared to healthy persons. As a predictive model for the best effect

on gastric cancer, we reported that the presence of a single G allele in the rs5745678 region of *HGF* could significantly affect the association risk of gastric cancer.

5.1. Conclusions

It is highly recommended that the expression pattern of *HGF* and the other relevant factors such as has-miR-320e be performed on a larger amount sample in breast and gastric cancers, which could represent valuable information about the effect of this mRNA and other biological factors on breast and gastric cancers. All in all, we represented that the frequency of G allele and GG genotype in the rs5745678 region of *HGF* has a significant increase in gastric cancer patients, as compared to healthy persons. As a predictive model for the best effect on gastric cancer, we reported that the presence of a single G allele in the rs5745678 region of *HGF* could significantly affect the association risk of gastric cancer.

Footnotes

Authors' Contribution: Fatemeh Keshavarzi supervised the project, created the idea of the study, and contributed to the critical revision of the manuscript for important intellectual content. Nahid Haghnazari supervised the project and follow-up. Mehrnoush Azadeh Jouneghani performed analysis and interpretation of data and contributed to the manuscript preparation. Sabrieh Amini and Zahra Hooshmandi were involved in data collection and patients' follow-up. All authors have read, critically revised, and approved the final manuscript.

Conflict of Interests: The authors declare no conflict of interest.

Ethical Approval: This research approved by the Ethics Committee of Sanandaj Branch, Islamic Azad University, Sanandaj, Iran (IR.IAU.SDJ.REC.1401.008). Link: ethics.research.ac.ir/EthicsProposalView.php?id=228667

Funding/Support: There was no funding/support.

References

1. Pennisi E. The Biology of Genomes. Disease risk links to gene regulation. *Science*. 2011;**332**(6033):1031. doi: 10.1126/science.332.6033.1031. [PubMed: 21617055].
2. Assad Samani L, Javadirad SM, Parsafar S, Tabatabaeian H, Ghaedi K, Azadeh M. TP53 rs1625895 is Related to Breast Cancer Incidence and Early Death in Iranian Population. *Indian J Clin Biochem*. 2019;**34**(4):485–9. doi: 10.1007/s12291-018-0774-6. [PubMed Central: PMC6801240].
3. Zheng J, Huang X, Tan W, Yu D, Du Z, Chang J, et al. Pancreatic cancer risk variant in LINC00673 creates a miR-1231 binding site and interferes with PTPN11 degradation. *Nat Genet*. 2016;**48**(7):747–57. doi: 10.1038/ng.3568. [PubMed: 27213290].
4. Kumar V, Westra HJ, Karjalainen J, Zhernakova DV, Esko T, Hrdlickova B, et al. Human disease-associated genetic variation impacts large intergenic non-coding RNA expression. *PLoS Genet*. 2013;**9**(1). e1003201. doi: 10.1371/journal.pgen.1003201. [PubMed: 23341781]. [PubMed Central: PMC3547830].
5. Mansouri Bidkani M, Tabatabaeian H, Parsafar S, Ghanei N, Fazilati M, Ghaedi K. ErbB4 receptor polymorphism 2368A>C and risk of breast cancer. *Breast*. 2018;**42**:157–63. doi: 10.1016/j.breast.2018.10.002. [PubMed: 30336339].
6. Zabihi N, Sadeghi S, Tabatabaeian H, Ghaedi K, Azadeh M, Fazilati M. The association between rs1972820 and the risk of breast cancer in Isfahan population. *J Cancer Res Ther*. 2017;**13**(1):26–32. doi: 10.4103/0973-1482.183202. [PubMed: 28508829].
7. Hubner RA, Houlston RS. Single Nucleotide Polymorphisms and Cancer Susceptibility. *The Molecular Basis of Human Cancer*. Springer; 2017. p. 231–9.
8. Dehghan Z, Sadeghi S, Tabatabaeian H, Ghaedi K, Azadeh M, Fazilati M, et al. ESR1 single nucleotide polymorphism rs1062577 (c.*3804T>A) alters the susceptibility of breast cancer risk in Iranian population. *Gene*. 2017;**611**:9–14. doi: 10.1016/j.gene.2017.02.016. [PubMed: 28216037].
9. Salimi Z, Sadeghi S, Tabatabaeian H, Ghaedi K, Fazilati M. rs11895168 C allele and the increased risk of breast cancer in Isfahan population. *Breast*. 2016;**28**:89–94. doi: 10.1016/j.breast.2016.05.007. [PubMed: 27262100].
10. Shastry BS. SNPs: impact on gene function and phenotype. *Methods Mol Biol*. 2009;**578**:3–22. doi: 10.1007/978-1-60327-411-1_1. [PubMed: 19768584].
11. McGeoch L, Saunders CL, Griffin SJ, Emery JD, Walter FM, Thompson DJ, et al. Risk Prediction Models for Colorectal Cancer Incorporating Common Genetic Variants: A Systematic Review. *Cancer Epidemiol Biomarkers Prev*. 2019;**28**(10):1580–93. doi: 10.1158/1055-9965.EPI-19-0059. [PubMed: 31292139]. [PubMed Central: PMC7610631].
12. Jang J, Ma SH, Ko KP, Choi BY, Yoo KY, Park SK. Hepatocyte Growth Factor in Blood and Gastric Cancer Risk: A Nested Case-Control Study. *Cancer Epidemiol Biomarkers Prev*. 2020;**29**(2):470–6. doi: 10.1158/1055-9965.EPI-19-0436. [PubMed: 31740519].
13. Czyz M. HGF/c-MET Signaling in Melanocytes and Melanoma. *Int J Mol Sci*. 2018;**19**(12). doi: 10.3390/ijms19123844. [PubMed: 30513872]. [PubMed Central: PMC6321285].
14. Tsuji S, Kawano S, Tsujii M, Fusamoto H, Kamada T. Roles of hepatocyte growth factor and its receptor in gastric mucosa. A cell biological and molecular biological study. *Dig Dis Sci*. 1995;**40**(5):1132–9. doi: 10.1007/BF02064211. [PubMed: 7729276].
15. Beviglia L, Matsumoto K, Lin CS, Ziober BL, Kramer RH. Expression of the c-Met/HGF receptor in human breast carcinoma: correlation with tumor progression. *Int J Cancer*. 1997;**74**(3):301–9. doi: 10.1002/(sici)1097-0215(19970620)74:3<301::aid-ijc12>3.0.co;2-e. [PubMed: 9221809].
16. Hecht M, Papoutsi M, Tran HD, Wilting J, Schweigerer L. Hepatocyte growth factor/c-Met signaling promotes the progression of experimental human neuroblastomas. *Cancer Res*. 2004;**64**(17):6109–18. doi: 10.1158/0008-5472.CAN-04-1014. [PubMed: 15342394].
17. Organ SL, Sierra J, Tsao M. Therapeutic potential of c-MET inhibitors: background and clinical data. *Clin Invest*. 2012;**2**(3):301–15. doi: 10.4155/cli.12.11.
18. Cui P, Zhao Y, Chu X, He N, Zheng H, Han J, et al. SNP rs2071095 in LincRNA H19 is associated with breast cancer risk. *Breast Cancer Res Treat*. 2018;**171**(1):161–71. doi: 10.1007/s10549-018-4814-y. [PubMed: 29737472].
19. Appleman LJ. MET signaling pathway: a rational target for cancer therapy. *J Clin Oncol*. 2011;**29**(36):4837–8. doi: 10.1200/JCO.2011.37.7929. [PubMed: 22042966].
20. Tsuta K, Kozu Y, Mimae T, Yoshida A, Kohno T, Sekine I, et al. c-MET/phospho-MET protein expression and MET gene copy number in non-small cell lung carcinomas. *J Thorac Oncol*. 2012;**7**(2):331–9. doi: 10.1097/JTO.0b013e318241655f. [PubMed: 22198430].

21. Edakuni G, Sasatomi E, Satoh T, Tokunaga O, Miyazaki K. Expression of the hepatocyte growth factor/c-Met pathway is increased at the cancer front in breast carcinoma. *Pathol Int*. 2001;**51**(3):172–8. doi: 10.1046/j.1440-1827.2001.01182.x. [PubMed: 11328532].
22. Schulte N, Ebert MP, Hartel N. Gastric Cancer: New Drugs - New Strategies. *Gastrointest Tumors*. 2014;**1**(4):180–94. doi: 10.1159/000380786. [PubMed: 26674336]. [PubMed Central: PMC4668795].
23. Zhang YW, Wang LM, Jove R, Vande Woude GF. Requirement of Stat3 signaling for HGF/SF-Met mediated tumorigenesis. *Oncogene*. 2002;**21**(2):217–26. doi: 10.1038/sj.onc.1205004. [PubMed: 11803465].
24. Pelicci G, Giordano S, Zhen Z, Salcini AE, Lanfrancone L, Bardelli A, et al. The motogenic and mitogenic responses to HGF are amplified by the Shc adaptor protein. *Oncogene*. 1995;**10**(8):1631–8. [PubMed: 7731718].
25. Fixman ED, Fournier TM, Kamikura DM, Naujokas MA, Park M. Pathways downstream of Shc and Grb2 are required for cell transformation by the tpr-Met oncoprotein. *J Biol Chem*. 1996;**271**(22):13116–22. doi: 10.1074/jbc.271.22.13116. [PubMed: 8662733].
26. Robert F, Pelletier J. Exploring the Impact of Single-Nucleotide Polymorphisms on Translation. *Front Genet*. 2018;**9**:507. doi: 10.3389/fgene.2018.00507. [PubMed: 30425729]. [PubMed Central: PMC6218417].
27. Dong G, Wang M, Gu G, Li S, Sun X, Li Z, et al. MACC1 and HGF are associated with survival in patients with gastric cancer. *Oncol Lett*. 2018;**15**(3):3207–13. doi: 10.3892/ol.2017.7710. [PubMed: 29435059]. [PubMed Central: PMC5778833].
28. Koh SA, Lee KH. HGF-mediated S100A11 overexpression enhances proliferation and invasion of gastric cancer. *Am J Transl Res*. 2018;**10**(11):3385–94. [PubMed: 30662594]. [PubMed Central: PMC6291695].
29. Wang C, Xi W, Ji J, Cai Q, Zhao Q, Jiang J, et al. The prognostic value of HGF-c-MET signaling pathway in Gastric Cancer: a study based on TCGA and GEO databases. *Int J Med Sci*. 2020;**17**(13):1946–55. doi: 10.7150/ijms.44952. [PubMed: 32788873]. [PubMed Central: PMC7415389].
30. Veenstra C, Perez-Tenorio G, Stelling A, Karlsson E, Mirwani SM, Nordenskoljd B, et al. Met and its ligand HGF are associated with clinical outcome in breast cancer. *Oncotarget*. 2016;**7**(24):37145–59. doi: 10.18632/oncotarget.9268. [PubMed: 27175600]. [PubMed Central: PMC5095065].
31. Choi J, Song N, Han S, Chung S, Sung H, Lee JY, et al. The associations between immunity-related genes and breast cancer prognosis in Korean women. *PLoS One*. 2014;**9**(7). e103593. doi: 10.1371/journal.pone.0103593. [PubMed: 25075970]. [PubMed Central: PMC4116221].
32. Sakai K, Takeda M, Okamoto I, Nakagawa K, Nishio K. Multiple regulatory mechanisms of hepatocyte growth factor expression in malignant cells with a short poly(dA) sequence in the HGF gene promoter. *Oncol Lett*. 2015;**9**(1):405–10. doi: 10.3892/ol.2014.2702. [PubMed: 25436000]. [PubMed Central: PMC4246610].
33. Muendlein A, Hubalek M, Geller-Rhomberg S, Gasser K, Winder T, Drexel H, et al. Significant survival impact of MACC1 polymorphisms in HER2 positive breast cancer patients. *Eur J Cancer*. 2014;**50**(12):2134–41. doi: 10.1016/j.ejca.2014.05.007. [PubMed: 24910416].