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Insulin-like Growth Factor Axis Gene Polymorphisms and Clinical Outcome in Pancreatic Cancer

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Abstract

Background and Aims—Insulin-like growth factor (IGF)-axis mediated signaling pathways play an important role in pancreatic cancer development and progression. We examined if IGF-axis gene variants are associated with clinical outcome in pancreatic cancer.

Methods—We retrospectively genotyped 41 single nucleotide polymorphisms (SNPs) from 10 IGF-axis genes in 333 patients with localized pancreatic adenocarcinoma and validated the findings in 373 patients with advanced disease. Associations between genotype and overall survival (OS) were evaluated using multivariable Cox proportional hazard regression models.

Results—*IGF1* *8470T>C, *IGF1R* IVS2+46329T>C, *IGFBP3* A32G, *IRS1* G972R in patients with localized disease; *IGF1R* IVS20-3431A>G, *IGF1R* T766T, *IGFBP3* -202A>C, *IRS1* IVS1+4315C>G, *IRS1* G972R in patients with advanced disease; and *IGF1R* T766T, *IGF2R* L252V, *IGFBP3* -202A>C, *IRS1* IVS1+4315C>G, *IRS1* G972R, *IRS2* IVS1+5687T>C in all patients were significantly associated with OS ($P \leq 0.007$). Two haplotypes containing the variant allele of either *IRS1* G972R or IVS1-10949G>A, and an *IRS2* haplotype predicted worse OS ($P \leq 0.002$). A significant correlation between increased number of unfavorable-genotypes and decreased OS was observed, patients with 0–1 (n=247), 2 (n=237), 3 (n=145), 4 (n=60) and 5–8 (n=17) unfavorable-genotypes had median survival time of 24.2, 16.4, 14.4, 9.6 and 7.4 months, respectively ($P < 0.001$). Several SNPs of *IGF1R*, *IGF2R*, and *IRS1* gene were significantly associated with tumor response to therapy and disease stage.

Conclusion—These data suggest that individual genetic variations in the IGF axis pathway may predict for worse survival in patients with pancreatic cancer. This information may identify population subgroups that could benefit from IGFR-targeted agents.

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Keywords

pancreatic cancer; Insulin-like growth factor (IGF)-axis; single nucleotide polymorphism; overall survival

INTRODUCTION

Pancreatic cancer is the fourth-leading cause of cancer mortality in the United States, with estimated 42,470 new cases and 35,240 deaths in 2009¹. As one of the most aggressive and treatment-resistant cancers, pancreatic cancer has the lowest 5-year survival rate among all malignancies¹. Despite the advances in understanding of the molecular mechanisms in pancreatic cancer development, molecular-targeted therapy has not been translated into reduced mortality or improved survival in this deadly disease². One of the clinical challenges is how to identify patients who are most likely to benefit from each type of molecular targeted therapies. Recent developments in pharmacogenetics have shown that patients' genetic variations in drug metabolism and DNA repair are associated with their individual responses to cytotoxic cancer therapies. However, few studies have investigated whether genetic variations in the genes/signaling pathways involved in molecular-targeted therapies are associated with heterogeneity in response to therapy or with patient survival. To fill this gap in knowledge, in the current study, we examined association of gene variants in the insulin-like growth factor (IGF) axis genes with clinical outcome of pancreatic cancer.

The IGF-axis consists of the ligands IGF1 and IGF2, IGF receptors IGF1R and IGF2R (IGF2 receptor), the IGF binding protein family (IGFBP1-6), and the proteins involved in intracellular signaling such as insulin-receptor substrate (IRS) family (IRS1-4). The *IGF1* gene encodes a protein similar in function and structure to insulin. *IGF2* is an imprinted gene expressed only from the paternally inherited allele. IGF-axis plays a pivotal role in maintaining tissue homeostasis, regulating cell proliferation, differentiation, and migration^{3, 4}. IGF-axis signaling pathway affects tumor biology via both metabolic and mitogenic pathways^{5, 6}. For example, insulin resistance contributes to the development of obesity- and diabetes-associated pancreatic cancer. Besides integration with the epidermal growth factor (EGF)/EGF receptor (EGFR), c-Jun-NH2-kinase (JNK)⁷⁻⁹ and met proto-oncogene (Met)¹⁰ signaling pathways, IGF-axis mediates activation of the RAS/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and phosphatidylinositol-3'-kinase (PI3K)/v-akt murine thymoma viral oncogene homolog (AKT)/mammalian target of rapamycin (mTOR) pathways to promote tumor development^{11, 12}. Because of its important role in regulating cell survival and death, the IGF-axis has emerged as a therapeutic target for cancer treatment^{13, 14}.

In this study, we tested the hypothesis that genetic variations in the IGF-axis modify clinical outcome in pancreatic cancer. We successfully genotyped 41 single nucleotide polymorphisms (SNPs) including 21 tagging SNPs and 20 functional SNPs of 10 IGF-axis genes (*IGF1*, *IGF2*, *IGF1R*, *IGF2R*, *IGFBP1*, *IGFBP3*, *IGFBP5*, *IRS1*, *IRS2*, and *IRS4*) which encodes the core components of IGF-axis upstream the PI3K/AKT/mTOR and RAS/MAPK/ERK signaling pathways (Fig. 1). We evaluated the association of the above genotypes with overall survival (OS) and tumor characteristics to explore a potential role of polymorphic variants of the IGF-axis genes in determining clinical outcome in pancreatic cancer.

PATIENTS AND METHODS

Patient Recruitment and Data Collection

The 706 patients included 154 patients with potentially resectable tumor enrolled onto phase II clinical trials of preoperative gemcitabine-based chemoradiation¹⁵ and 552 patients recruited in a case-control study conducted at The University of Texas M. D. Anderson Cancer Center from February 1999 through May 2007 and follow-up to August 2009. The eligibility criteria for patient selection were: 1) having been diagnosed with a pathologically confirmed pancreatic ductal adenocarcinoma; 2) having an available DNA sample. All patients signed an informed consent for medical record review and gave a DNA sample. The study was approved by M. D. Anderson Cancer Center's Institutional Review Board and was conducted in accordance with all current ethical guidelines.

We reviewed patients' medical records and retrieved information on: date of patient diagnosis, date of death or last follow-up, tumor resection status, clinical tumor stage, patient performance status, tumor site, tumor size, tumor differentiation, serum markers for liver, kidney and pancreas functions (biochemical index), and level of serum carbohydrate antigen 19-9 (CA19-9) at diagnosis. Clinical tumor staging followed the objective computed tomography (CT) criteria: localized or potentially resectable tumors are defined as (1) no evidence of extrapancreatic disease; (2) no evidence of tumor extension to the superior mesenteric artery (SMA) or celiac axis; and (3) no evidence of occlusion of the superior mesenteric vein (SMV) or SMV–portal vein (PV) confluence. Tumor abutment and encasement of the SMV, in the absence of vessel occlusion or extension to the SMA was considered resectable. Locally advanced tumors are those unresectable but without distant metastasis. Tumor response to preoperative therapy by CT at the time of restaging was evaluated among patients who had a localized tumor and received preoperative chemoradiotherapy only. Tumor margin and lymph node status were evaluated among patients with resected tumors only. The clinical information was double-checked by different researchers. Dates of death were obtained and cross-checked using at least one of the following sources: M. D. Anderson Cancer Center's tumor registry, inpatient medical records, or the United States Social Security Death Index (www.deathindexes.com/ssdi.html). OS was calculated from the date of diagnosis to the date of death or last follow-up.

DNA Extraction, SNP Selection and Genotyping

DNA was extracted from peripheral lymphocytes using Qiagen DNA isolation kits (Valencia, CA). The genes, nucleotide substitutions, function, reference SNP identification numbers, and minor allele frequency (MAF) of the 45 SNPs examined are summarized in Table 1. Twenty-three tagging SNPs were selected using the SNPbrowser software (Applied Biosystems, www.allsnps.com/snpbrowser) with a cutoff of 0.8 for r^2 and a $MAF \geq 10\%$ in Caucasians from the HapMap Project database (www.hapmap.org). We chose 22 functional SNPs located in coding region (nonsynonymous or synonymous) or untranslated region (UTR) with a $MAF \geq 1\%$ in Caucasians. The protein sequences, structures, homology models, mRNA transcripts, and predicted functions for the examined SNPs were evaluated using F-SNP (Queen's University, Kingston, Ontario, Canada)¹⁶. Genotyping used the mass spectroscopy-based MassArray (Sequenom, Inc, San Diego, CA) and the Taqman method. Genotyping was successful in 41 of the 45 SNPs (Table 1). Randomly selected 20% of the total samples were genotyped in duplicate, showed 99.9% concordance. The inconsistent data were excluded from final analysis.

Statistical Analysis

The distribution of genotypes was examined for Hardy-Weinberg equilibrium with the goodness-of-fit chi-squared test. Genotype and allele frequency of the SNPs were determined by direct gene counting. Haplotype diversity and linkage disequilibrium index (Lewontin's D' and r^2) were calculated by SNPalyze software (DYNACOM Co, Ltd, Mobara, Japan). The homozygous and heterozygous genotypes were combined if the frequency of the homozygote was very low or if both genotypes had the same trend of effect (e.g., shorter OS compared with the referent group). The association between genotype or haplotype and OS was estimated using the Kaplan-Meier method and the log-rank test. The median follow-up time was computed using censored observations only. Hazard ratios (HR) and 95% CI were estimated using the multivariate Cox regression proportional hazards model adjusting for sex, age, race, clinical stage, tumor resection, tumor size and site, serum CA19-9 level and performance status at diagnosis. The association of genotype with tumor response to therapy or tumor differentiation was evaluated using logistic regression adjusting for the clinical factors. All statistical testing used SPSS software (SPSS Inc, Chicago, IL). The false discovery rate (FDR) was estimated using the Beta-Uniform Mixture (BUM) approach¹⁷. The model is used to obtain an estimated distribution expressed as a mixture of null and alternative densities. Given a threshold of significance, the estimated distribution is partitioned into regions corresponding to the occurrences of false positives¹⁷. To evaluate the association of genotype and OS, for 70 comparisons in 41 SNPs (42 in dominant and 28 in recessive inheritance modes, with ≥ 20 patients per group), we found a P value of 0.007 corresponded to an FDR of 10%. Any $P \leq 0.007$ in the genotype analysis was considered statistically significant.

RESULTS

Patients' Characteristics

The patients' characteristics and clinical predictors for OS are described in Table 2. The 706 patients were divided into a training set of 333 patients with localized tumors and a validation set of 373 patients with advanced disease (locally advanced and metastatic tumors). The training set included 154 patients who were treated on protocol and 179 patients who were treated off protocol with gemcitabine-based chemoradiation. Two hundred seventy five patients achieved tumor resection. The 373 patients with advanced tumor included 211 patients with locally advanced disease and 162 patients with metastatic disease. At the end of the study, 568 of the 706 patients (80.5%) were dead. The median follow-up time was 46.0 months for the living patients. The median survival time (MST) was 17.2 months (95% CI, 15.8–18.5) for the 706 patients. Patients with unresected tumor, advanced disease stage, an elevated CA19-9 serum level, a tumor size >2 cm, tumors located in the body or tail of the pancreas, poorly differentiated tumors, poor response to therapy, poor performance status, abnormal biochemical index were significantly associated with reduced OS.

Genotype Distribution and Allele Frequency

The observed MAF among the patients were comparable to the reported frequency in general population (Table 1). The distribution of 41 SNPs followed the Hardy-Weinberg equilibrium ($P > 0.05$) except for *IRS2* Ex2+750A>G, *IRS4* A20A ($P = 0.001$). Genotype distribution differed according to sex and race, e.g., men had a higher frequency of *IRS1* IVS1-10949GG and *IRS2* P829P CC/TC genotype than women had ($P \leq 0.007$); white patients had a higher frequency of *IGF2* *4352AA and *IGF1R* IVS2-35846TT/TC genotype than non-white patients had ($P < 0.001$). The haplotype diversity and the linkage disequilibrium data of the 41 SNPs are summarized in supplemental Tables 1 and 2, respectively.

Association of Genotype with Overall Survival

The association of each genotype with OS was first evaluated in the training set of 333 patients with resectable tumors and then analyzed in the validation set of 373 patients with advanced disease (Table 3). To increase power, data from the training set and validation set was also analyzed in combination. *IGF1* *8470T>C, *IGF1R* IVS2+46329T>C, *IGFBP3* A32G, and *IRS1* G972R in patients with localized disease; *IGF1R* IVS20-3431A>G, *IGF1R* T766T, *IGFBP3* -202A>C, *IRS1* IVS1+4315C>G and *IRS1* G972R in patients with advanced disease; and *IGF1R* T766T, *IGF2R* L252V, *IGFBP3* -202A>C, *IRS1* IVS1+4315C>G, *IRS1* G972R, and *IRS2* IVS1+5687T>C in all patients were significantly associated with OS after adjusting for clinical factors ($P \leq 0.007$). Two of the four SNPs identified in the training set, i.e. *IGF1* *8470T>C and *IGFBP3* A32G genotype showed significant associations with OS in patients with localized disease but not in patients with advanced disease. The *IGF1R* T766T, *IGFBP3* -202A>C, *IRS1* G972R and *IRS2* IVS1+5687T>C genotype showed consistent associations with OS in both training set and validation set and in the combined dataset. The *IRS1* IVS1+4315C>G GG genotype and the *IGF2R* L252V GG genotype was associated with much reduced OS in different patient groups but the frequency of these homozygous variants was extremely low (<2%).

We analyzed the combined effect of individual SNPs on OS by including the unfavorable genotypes that were associated with reduced survival and had a $P < 0.05$ in the multivariable Cox regression model, i.e. *IGF1R* IVS2+46329T>C TC, T766T CT/TT, *IGF2R* L252V GG/CG, *IGFBP3* A32G GC/CC, -202A>C CC, *IRS1* IVS1+4315C>G CG/GG, G972R GA/AA, and *IRS2* IVS1+5687T>C CT/CC. A strong association between having two or more unfavorable-genotypes and reduced OS in all patients was observed. Patients with 0–1 (n=247), 2 (n=237), 3 (n=145), 4 (n=60) and 5–8 (n=17) unfavorable-genotypes had MSTs of 24.2, 16.4, 14.4, 9.6 and 7.4 months, respectively ($P < 0.001$). Compared to patients with 0–1 unfavorable-genotype, for those carrying 2, 3, 4 or 5–8 unfavorable-genotypes, adjusted HR (95% CI) were 1.35 (1.09–1.67), 1.74 (1.38–2.20), 3.15 (2.28–4.34) and 6.79 (3.80–12.1), respectively.

Associations of Haplotype Diversity with OS

Eight haplotypes of *IGF2R*, *IGFBP3*, *IGFBP5*, *IRS1* and *IRS2* were associated with reduced or better OS in 706 patients in Cox regression after adjusting for clinical factors ($P \leq 0.05$, Table 4). Two haplotypes CAGG and GGGG containing *IRS1* G972R A allele and IVS1-10949G>A G allele, respectively, and one haplotype (AGCGCC) of *IRS2* containing Ex2+750A>G A allele, G1057D G allele and IVS1+5687T>C C allele remained significant at the level of FDR < 10% ($P \leq 0.007$).

Associations of Genotype with Tumor Characteristics

We examined the associations between genotypes with response to therapy, tumor stage and tumor resection status (Table 5). *IGF1R* IVS2-70184A>G, *IGF2R* L2222L and *IRS1* G972R were associated with a poor tumor response to preoperative chemoradiotherapy and remained as significant predictors in logistic regression after adjusting for clinical factors ($P \leq 0.007$). *IGF1* Ex4+2776G>A, *IGF1R* IVS2-70184A>G, and *IRS2* IVS1+5687T>C correlated with tumor stage ($P \leq 0.007$). *IGF1* Ex4+2776G>A, *IGF1R* IVS2-70184A>G, T766T, and *IRS2* IVS1+5687T>C correlated with tumor resectability ($P \leq 0.007$).

DISCUSSION

In this study, we found significant associations between IGF-axis gene variants and clinical outcome of patients with pancreatic cancer. *IGF1* *8470T>C, *IGF1R* IVS2+46329T>C, *IGFBP3* A32G, *IRS1* G972R in patients with localized disease; *IGF1R* IVS20-3431A>G,

IGF1R T766T, *IGFBP3* -202A>C, *IRS1* IVS1+4315C>G, *IRS1* G972R in patients with advanced disease; and *IGF1R* T766T, *IGF2R* L252V, *IGFBP3* -202A>C, *IRS1* IVS1+4315C>G, *IRS1* G972R, *IRS2* IVS1+5687T>C in all patients were significantly associated with OS ($P \leq 0.007$). A strong association between having two or more of the predictive genotypes and having poorer OS was observed among all patients. Significant associations between genotypes and response to preoperative chemoradiotherapy, tumor stage, and tumor resectability were detected as well. For the first time to our knowledge, we have demonstrated a potential role of gene variations along the IGF-axis in determining the clinical outcome of patients with pancreatic cancer.

Among the 41 SNPs, the *IGF1* *8470T>C TT and *IGFBP3* A32G CC genotypes were predictors for worse or better OS, respectively in patients with localized tumors only. A recent study has shown that these two gene variants were significantly associated with circulating level of their coded proteins¹⁸. *IGFBP3* prolongs the half-life of IGFs by binding with the circulating IGFs and altering their interactions with cell surface receptors. As a regulator of cell survival, *IGFBP-3* is involved in cytokine-mediated apoptosis in insulin-secreting cells¹⁹. Because *IGFBP3* plays an important role in regulating the bioavailability of *IGF1*²⁰, these observations suggest that the *IGF1* level or the ratio of *IGF1/IGFBP3* may play a more critical role in tumor progression in the early stages of pancreatic cancer. This would be a beneficial area for further research.

In patients with advanced diseases and in all patients, the *IGFBP3* promoter region SNP -202A>C, which is in LD with the A32G SNP, was significantly associated reduced OS. None of the *IGFBP1* and *IGFBP5* SNPs in this study showed significant association with OS, even though both genes have been suggested to play a role in pancreatic cancer^{21–23}.

The *IGF1R* T766T, *IRS1* G972R and *IRS2* IVS1+5687T>C genotype showed consistent associations with OS in patients with localized disease and in patients with advanced disease. These genotypes were also significantly associated with tumor response to therapy or tumor stage and resectability. The *IGF1R* T766T is a synonymous SNP without changing amino acid. Although functional analysis by bioinformatics¹⁶ predicted that the variant allele may lead to alternative mRNA splicing and abnormal protein functions¹⁶, no direct evidence is available at present. The *IRS1* G972R genotype and 972R-containing haplotype were predictive for reduced OS in all patients. The functional effect of the *IRS1* 972R variant has been investigated previously. Cells with the 972R variant allele have reduced insulin secretion^{24, 25} and insulin sensitivity²⁶, impaired glucose transport and glycogen synthesis²⁷, increased insulin resistance²⁶, and impaired β -cell survival^{24, 28}. *IRS1* 972R can phosphorylate and upregulate AKT in a PI3K-independent manner, possibly through stress and hypoxia, to activate downstream mTOR pathways to promote cell proliferation and survival²⁹. These factors may help explain the association between *IRS1* 972R variant and reduced OS in pancreatic cancer. Our data suggest the *IRS1* G972R genotype may be a useful marker to help patient selection for the *IGF1R*-targeted therapy. Activated *IGF1R* phosphorylates the IRSs to interact with PI3K/AKT and RAS/MAPK/ERK signaling transducers, send mitogenic, anti-apoptotic, and dedifferentiation signals^{7–10}. Activation of the signaling pathways plays an important role in promoting pancreatic cancer development and progression^{30–33}. *IRS2* IVS1+5687C allele was predictive for worse OS in all patients. This intronic SNP is in linkage disequilibrium with *IRS2* G1057D, a nonsynonymous SNP that was predicted to affect splicing, transcriptional regulation, and post-translational modification (16); and *IRS2* Ex2+750A>G, a 3'UTR SNP. Even though the latter two SNPs were not individually significantly associated with OS, *IRS2* AGCGCC haplotype containing the *IRS2* Ex2+750A, G1057, IVS1+5687C allele showed strong associations with reduced OS.

IGF2R is a receptor for both IGF2 and mannose 6-phosphate (M6P), which is a tumor suppressor. It reduces IGF2 bioactivity by sequestering it away from IGF1R⁶. Loss of IGF2R is associated with tumor progression through impaired trafficking of lysosomal enzymes, reduced availability of TGF- β , inhibited apoptosis, and activated proteolytic processes³⁴. We observed that the homozygous mutant of *IGF2R* L252V was significantly associated with OS in all patients. The variant allele of this nonsynonymous SNP was predicted to be deleterious to the protein-coding 3D conformation (through causing the loss of hydrophobic effect and creation of a cavity) and/or regulate gene splicing¹⁶. However, there were only 14 patients with the homozygous mutant genotype in this study; this association needs to be confirmed in a larger study.

We did not observe any association between SNPs in the *IGF2* gene and clinical outcomes in pancreatic cancer. The lack of such an association could be due to the limited number of SNPs examined or the relatively less-important role of IGF2 in tumor progression.

Because of the large number of SNPs examined in this study, we considered a $P \leq 0.007$ as significant to keep the FDR < 10%. Many of the SNPs had $P < 0.05$ but > 0.007 , however, when we calculated the number of unfavorable-genotypes, we observed dramatic combined-genotype association with OS, suggesting that genes involved in the IGF-axis function synergistically and that the combined effect of several genes may have a greater influence on the phenotype, e.g., OS, than do individual genes.

The strengths of this study include a large sample size, detailed clinical information, and hypothesis-driven selection of genes. The limitations of this study included the limited number of genes and SNPs examined and the potential false-positive findings related to multiple comparisons. Nevertheless, the study findings provide supporting evidence for the importance of genes from the IGF-axis in pancreatic cancer. Because of the difficulties in obtaining the target tissues for gene expression profiling in most patients with pancreatic cancer, the genetic markers described in this study, which was determined in a DNA sample collected non-invasively, along with well characterized phenotypic significance has great clinical implications. For example, these genotypes might be useful in selecting patients for the IGF1R-targeted therapy, i.e. those carrying the variant alleles that are associated with reduced survival or poor response to therapy might benefit more from such treatment. Whether these genetic markers are predictive to response to IGF1R inhibitors are under current investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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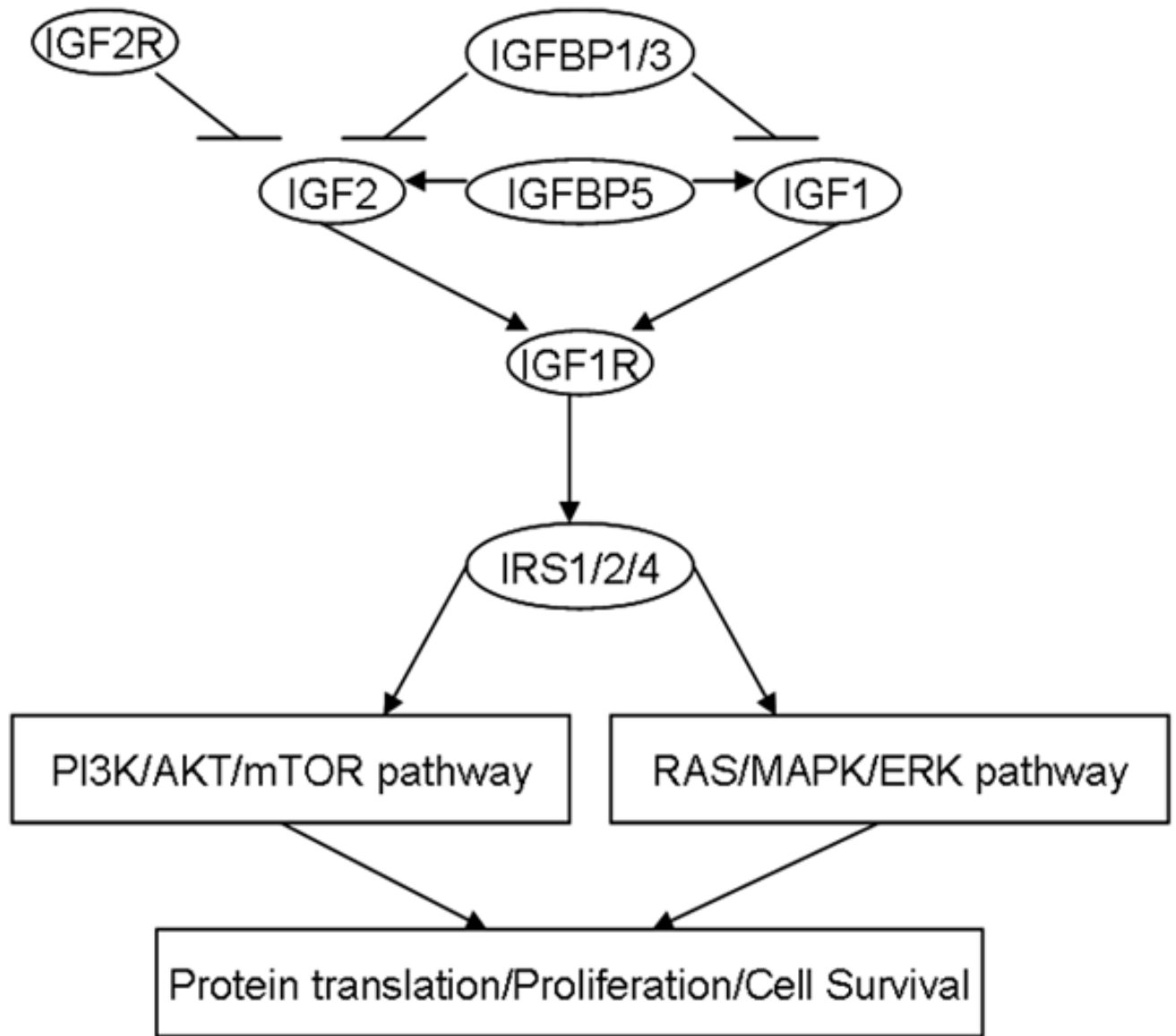


Fig. 1. Selected IGF-axis genes that encode core components of IGF-axis upstream the PI3K/AKT/mTOR and RAS/MAPK/ERK pathway. The ligands IGF1 and IGF2 bind to the receptor IGF1R at the cellular level. The bioavailability of IGFs is decreased by IGFBP1 and IGFBP3, but increased by IGFBP5. IGF2 is inhibited by negative feedback of IGF2R, which blocks the signal transduction. IGF1R regulate the downstream PI3K/AKT/mTOR and RAS/MAPK/ERK pathways via activation of IRS proteins.

Table 1

SNPs Examined and Allele Frequency

Gene	Chromosome	SNP	Function	RS#	Minor Allele Frequency	
					Observed	Reported*
<i>IGF1</i>	12q22-q23	-1409T>C	5' Flank	35767	0.176	0.161
		IVS2+10605A>G	Intron	7956547	0.255	0.25
		IVS2+12719G>C	Intron	2195239	0.252	0.235
		IVS2+25185C>T	Intron	10735380	0.296	0.285
		*8470T>C	3' Flank	2946834	0.335	0.313
		*10212C>A	3' Flank	10860862	0.187	0.175
		Ex4-517G>A	3'UTR	6219	failed	0.076
		Ex4+2776G>A	3'UTR	6214	0.30	0.421
		Ex4-2894G>A	3'UTR	1063599	failed	0.16
		IVS2-70184A>G	Intron	2684761	0.39	0.415
<i>IGF1R</i>	15q26.3	IVS2-35846T>C	Intron	1879612	0.444	0.42
		IVS2+46329T>C	Intron	8041224	0.401	0.40
		IVS11+538G>A	Intron	4966044	0.381	0.392
		IVS15+1950A>G	Intron	7166558	0.411	0.392
		IVS17-598C>T	Intron	2684799	0.407	0.492
		IVS20-3431A>G	Intron	12437963	0.181	0.142
		IVS20-797G>T	Intron	2872060	0.482	0.457
		Ex11+97C>T	T766T	3743262	0.058	0.058
		Ex16-58G>A	E1043E	2229765	0.439	0.419
		Ex21+316C>T	Y1346Y	17847203	0.063	0.056
<i>IGF2</i>	11p15.5	*4352A>G	3' Flank	11042751	0.189	0.29
		IVS2+384C>T	Intron	734351	failed	0.387
<i>IGF2R</i>	6q26	Ex6-23C>G	L252V	8191754	0.124	0.142
		Ex9+5A>G	S350S	1570070	0.348	0.342
		Ex16+88A>G	T713T	998075	0.483	0.452
		Ex45+11C>T	L2222L	1803989	0.107	0.097
<i>IGFBP1</i>	7p13-p12	Ex4+111A>G	I253M	4619	0.40	0.36

Gene	Chromosome	SNP	Function	RS#	Minor Allele Frequency	
					Observed	Reported*
IGFBP3	7p13-p12	Ex1+227C>G	A32G	2854746	0.39	0.403
		Ex5-411A>T	3'UTR	6670	0.211	0.197
		Ex2+70A>C	H164P	9282734	0.005	0.01
IGFBP5	2q33-q36	-202A>C	Promoter	2854744	0.48	0.476
		IVS1-6727A>C	Intron	7420849	0.43	0.45
		Ex2+75C>T	R138W	11575194	0.043	0.05
IRSI	2q36	IVS1-10949G>A	Intron	35802832	0.045	0.12
		IVS1+4315C>G	Intron	2288586	0.07	0.097
		IVS1+14180A>T	Intron	1025333	failed	0.15
		Ex1-840G>A	G972R	1801278	0.076	0.07
IRS2	13q34	Ex1-2217G>C	A512P	1801276	0.026	0.06
		IVS1+166T>C	Intron	9521511	0.391	0.43
		IVS1+2498G>A	Intron	7981705	0.169	0.175
		IVS1+5687T>C	Intron	1974134	0.299	0.44
		Ex1-1526C>T	P829P	12853546	0.229	0.177
		Ex1-843G>A	G1057D	1805097	0.315	0.21
IRS4	Xq22.3	Ex2+750A>G	3'UTR	2289046	0.362	0.379
		Ex1+137G>A	A20A	2073115	0.18	0.167

SNP, single nucleotide polymorphism; RS#, reference SNP identification number; UTR, untranslated region.

*The reported minor allele frequency was from the SNP500Cancer and NCBI dbSNP databases.

Table 2

Patient's Characteristics and Clinical Predictors for Overall Survival

Variable	No. of Patients	No. of Deaths	MST (months)	P (log-rank)
Gender				0.48
Female	281	235	17.5	
Male	425	333	16.8	
Age (years)				0.22
≤ 50	90	80	16.4	
51–60	181	142	18.4	
61–70	272	213	17.2	
> 70	163	133	16.5	
Race				0.63
White	624	502	16.6	
Hispanic	43	35	16.8	
Black	27	21	20.2	
Other	12	10	17.3	
Clinical Stage				<0.001
Localized	333	237	28.5	
Locally Advanced	211	188	14.7	
Metastatic	162	143	9.2	
Performance Status				<0.001
0	148	106	31.0	
1	483	394	16.0	
2–3	75	68	9.5	
Tumor Size (cm)				<0.001
≤ 2	134	94	27.5	
> 2	572	474	15.3	
Tumor Site				0.001
Head	511	411	18.2	
Non-head	195	157	13.7	
CA 19-9 (U/ml)				<0.001
≤ 47	161	107	32.1	
48–500	291	237	18.0	
500–1000	73	60	14.5	
> 1000	181	164	10.6	
Tumor Differentiation[†]				<0.001
Well to Moderate	355	261	26.2	
Poorly	139	120	12.0	
Tumor Response to Therapy[‡]				<0.001
PR/SD	225	146	35.8	
PD	36	35	9.3	
Tumor Resection				<0.001

Variable	No. of Patients	No. of Deaths	MST (months)	<i>P</i> (log-rank)
Yes	275	179	35.9	
No	431	389	12.0	
Biochemical Index*				0.008
0–2	303	230	18.9	
3–6	362	302	16.0	
7–9	41	36	12.9	

MST, median survival time; CI, confidence interval; HR, hazard ratio; CA 19-9, carbohydrate antigen 19-9; PR, partial response; SD, stable disease; PD, progressive disease.

[†]This information was available for patients with cytological diagnosis only.

[‡]Tumor response to therapy was evaluated in patients received preoperative chemoradiotherapy by CT at restaging. Node and margin status was evaluated in surgical patients only.

* Biochemical index represents the number of serum markers with abnormal value. The markers include aspartate aminotransferase, lactic dehydrogenase, alkaline phosphatase, alanine aminotransferase, amylase, creatinine, hemoglobin, albumin, bilirubin, and fasting glucose.

Table 3

Association of Genotype with Overall Survival

Genotype	Training				Validation				Combined dataset			
	No. of Patients/No. of Deaths	MST	HR [†] (95%CI)	P [†]	No. of Patients/No. of Deaths	MST	HR [†] (95%CI)	P [†]	MST	HR [†] (95% CI)	P [†]	
<i>IGF1</i> *8470T>C												
CC	150/94	28.7	1.0		161/145	11.4	1.0		16.5	1.0		
CT	142/110	29.8	1.38 (1.04–1.83)	.02	169/149	12.5	0.97 (0.77–1.22)	.80	17.7	1.08 (0.90–1.29)	.39	
TT	41/33	25.7	2.01 (1.33–3.03)	<.001	38/33	13.5	0.89 (0.60–1.31)	.55	18.0	1.22 (0.92–1.61)	.16	
<i>IGF1R</i> IVS2+46329T>C												
CC	140/89	33.3	1.0		120/109	14.5	1.0		20.5	1.0		
TC	144/112	24.3	1.49 (1.12–1.99)	.006	178/161	11.4	1.23 (0.96–1.58)	.11	14.4	1.34 (1.11–1.62)	.002	
TT	49/36	31.0	1.06 (0.71–1.58)	.76	72/58	14.5	0.94 (0.68–1.31)	.73	18.7	1.06 (0.83–1.37)	.62	
CC/TT vs. TC			1.47 (1.13–1.91)	.004			1.26 (1.01–1.57)	.04		1.32 (1.11–1.56)	.01	
<i>IGF1R</i> IVS20-3431A>G												
AA	232/170	26.4	1.0		240/211	13.2	1.0		17.6	1.0		
GA	89/59	33.6	0.76 (0.56–1.03)	.07	122/109	10.4	1.36 (1.07–1.72)	.01	15.3	1.12 (0.94–1.35)	.21	
GG	12/8	16.2	1.11 (0.54–2.29)	.78	10/10	4.9	1.65 (0.85–3.19)	.14	12.0	1.30 (0.80–2.10)	.29	
AA vs. GG/GA			0.78 (0.58–1.04)	.09			1.40 (1.11–1.77)	.004		1.14 (0.95–1.36)	.15	
<i>IGF1R</i> T766T												
CC	301/211	30.6	1.0		327/287	12.6	1.0		18.2	1.0		
CT	31/25	15.5	1.59 (1.04–2.45)	.03	43/41	8.0	1.70 (1.20–2.39)	.002	10.3	1.61 (1.22–2.12)	<.001	
TT	1/1	9.2	2.60 (0.32–21.1)	.37	3/3	7.3	3.50 (1.02–12.02)	.046	7.3	2.50 (0.89–7.02)	.08	
CC vs. CT/TT			1.61 (1.06–2.46)	.02			1.77 (1.27–2.47)	<.001		1.66 (1.28–2.16)	<.001	
<i>IGF2R</i> L252V												
CC	256/175	30.4	1.0		288/261	12.0	1.0		17.5	1.0		
CG	72/57	26.2	1.20 (0.88–1.64)	.24	75/61	13.6	0.86 (0.65–1.15)	.32	16.4	1.01 (0.82–1.24)	.95	
GG	5/5	11.0	2.92 (1.09–7.77)	.03	9/8	11.1	1.67 (0.82–3.42)	.16	11.0	2.36 (1.31–4.23)	.004	
CC vs. GG/CG			2.81 (1.06–7.47)	.038			1.73 (0.85–3.52)	.13				
<i>IGF2R</i> S350S												
AA	156/97	36.4	1.0		156/143	12.4	1.0		18.7	1.0		
AG	139/107	21.7	1.28 (0.96–1.70)	.09	158/139	12.0	1.00 (0.78–1.26)	.97	16.5	1.11 (0.92–1.33)	.26	

Genotype	Training				Validation				Combined dataset			
	No. of Patients/No. of Deaths	MST	HR [†] (95%CI)	P [†]	No. of Patients/No. of Deaths	MST	HR [†] (95%CI)	P [†]	MST	HR [†] (95% CI)	P [†]	
GG	38/33	22.9	1.72 (1.13–2.61)	.01	59/49	12.1	1.02 (0.74–1.42)	.89	15.1	1.25 (0.97–1.62)	.09	
<i>IGFBP3 A32G</i>												
GG	137/108	22.9	1.0		132/114	11.1	1.0		16.4	1.0		
GC	145/99	29.8	0.71 (0.54–0.93)	.01	176/157	13.9	0.89 (0.69–1.13)	.33	17.9	0.81 (0.67–0.97)	.02	
CC	49/30	34.8	0.58 (0.38–0.87)	.009	65/60	11.8	1.08 (0.78–1.48)	.65	15.3	0.88 (0.69–1.13)	.33	
GG vs. CC/GC			0.67 (0.52–0.87)	.003			0.93 (0.74–1.18)	.55		0.83 (0.70–0.98)	.029	
<i>IGFBP3 -202A>C</i>												
CC	72/57	24.5			85/73	10.3	1.0		14.7			
AC	173/119	30.1	0.72 (0.52–0.99)	.046	195/175	13.4	0.65 (0.49–0.87)	.003	17.8	0.68 (0.55–0.84)	<.001	
AA	88/61	28.5	0.69 (0.48–0.99)	.049	93/83	12.5	0.70 (0.50–0.98)	.039	17.6	0.71 (0.55–0.91)	.006	
CC vs. AA/AC			0.71 (0.52–0.96)	.02			0.67 (0.51–0.87)	.003		0.69 (0.57–0.84)	<.001	
<i>IRS1 IVS1+4315C>G</i>												
CC	284/199	30.4	1.0		327/289	12.0	1.0		17.3	1.0		
CG	46/35	18.2	1.39 (0.96–2.02)	.08	42/38	12.3	1.15 (0.8–1.65)	.44	17.2	1.22 (0.94–1.57)	.13	
GG	3/3	9.2	4.26 (1.25–14.5)	.02	2/2	3.2	22.8 (5.21–99.4)	<.001	3.5	5.63 (2.25–14.0)	<.001	
CC vs. CG/GG			1.47 (1.02–2.11)	.03			1.23 (0.87–1.74)	.24		1.29 (1.01–1.65)	.04	
<i>IRS1 G972R</i>												
GG	292/204	30.4	1.0		314/277	12.7	1.0		18.2	1.0		
GA	38/30	12.9	1.65 (1.11–2.46)	.01	54/51	8.5	1.42 (1.05–1.93)	.02	10.1	1.50 (1.18–1.91)	.001	
AA	3/3	6.2	3.25 (1.01–10.4)	.047	5/3	3.8	1.43 (0.44–4.58)	.55	6.2	2.26 (1.00–5.11)	.05	
GG vs. GA/AA			1.74 (1.19–2.54)	.005			1.49 (1.10–2.01)	.009		1.53 (1.21–1.94)	<.001	
<i>IRS2 IVS1+5687T>C</i>												
TT	205/140	31.2	1.0		132/112	13.6	1.0		20.7	1.0		
CT	101/80	25.1	1.42 (1.06–1.89)	.01	179/164	12.0	1.24 (0.97–1.59)	.08	15.1	1.24 (1.03–1.49)	.02	
CC	20/14	28.5	1.56 (0.89–2.73)	.12	43/39	10.1	1.45 (0.99–2.11)	.05	12.5	1.46 (1.07–1.98)	.01	
TT vs. CT/CC			1.44 (1.09–1.89)	.009	132/112	11.8	1.28 (1.01–1.62)	.04		1.27 (1.07–1.52)	.007	
<i>IRS4 A20A</i>												
GG	244/167	30.1			275/238	12.2			17.5			
GA	43/35	28.1	1.20 (0.80–1.80)	.37	54/50	11.8	1.31 (0.81–1.59)	.48	14.7	1.13 (0.87–1.46)	.35	
AA	37/28	25.0	1.55 (1.01–2.39)	.048	41/40	12.8	1.10 (0.77–1.56)	.61	18.1	1.25 (0.96–1.64)	.10	

Genotype	Training			Validation			Combined dataset				
	No. of Patients/No. of Deaths	MST	HR [‡] (95%CI)	P [‡]	No. of Patients/No. of Deaths	MST	HR [‡] (95%CI)	P [‡]	MST	HR [‡] (95% CI)	P [‡]
GG vs. GA/AA			1.52 (1.00-2.34)	.05			1.11 (0.86-1.44)	.41		1.19 (0.97-1.44)	.09

MST, median survival time (months); CI, confidence interval; HR: hazard ratio; CA19-9, carbohydrate antigen 19-9.

[‡]HR was estimated from multivariate Cox regression models including sex, race, clinical stage, tumor resection, CA 19-9, biochemical index and performance status

Table 4

Association of Haplotype Diversity with OS in All Patients

Haplotype*	Frequency	MST	HR [†] (95% CI)	P
<i>IGF2R</i>				
ACAC	0.3287	18.7	1.0	
GTGC	0.0577	16.5	1.28 (1.00–1.64)	0.05
<i>IGFBP3</i>				
CGAT	0.3748	16.3	1.0	
ACAT	0.2825	17.7	0.87 (0.77–0.99)	0.04
<i>IGFBP5</i>				
CA	0.538	17.5	1.0	
TC	0.01	5.0	2.73 (1.02–7.34)	0.046
<i>IRS1</i>				
CGGG	0.8243	18.1	1.0	
CAGG	0.072	10.3	1.41 (1.13–1.76)	0.002
GGGG	0.033	13.3	1.76 (1.28–2.42)	<0.001
<i>IRS2</i>				
GACGTC	0.2399	18.5	1.0	
AGCGCT	0.0595	14.3	1.35 (1.02–1.77)	0.03
GGCGTC	0.0439	14.7	1.43 (1.04–1.96)	0.03
AGCGCC	0.0405	14.3	1.67 (1.21–2.29)	0.001

MST, median survival time (months); CI, confidence interval; HR: hazard ratio; CA19-9, carbohydrate antigen 19-9 HR values were from multivariate Cox regression models including sex, race, clinical stage, tumor resection, CA 19-9, biochemical index and performance status.

* Haplotype of *IGF2R* T713T, L2222L, S350S, L252V; *IGFBP3* -202A>C, A32G, H164P, Ex5-411A>T; *IGFBP5* R138W, IVS1-6727A>C; *IRS1* IVS1+4315C>G, G972R, IVS1-10949G>A, A512P; *IRS2* Ex2+750A>G, G1057D, P829P, IVS1+2498G>A, IVS1+5687T>C, IVS1+166T>C. Those haplotypes with $P>0.05$ in Cox regression models were not shown.

Table 5

Association of Genotypes with Tumor Characteristics in All Patients

Variable	Genotype	No. (%)	No. (%)	P (χ^2)	OR [†] (95% CI)	P [‡]
Response to Therapy						
PR/SD		PD				
<i>IGF1R</i> IVS2-70184A>G						
	AA	108 (90.8)	11 (9.2)	.001	1.0	
	GA/GG	116 (75.8)	37 (24.2)		4.81 (2.02–11.4)	<.001
<i>IGF1R</i> IVS15+1950A>G						
	GG	51 (98.1)	1 (1.9)	.001*	1.0	
	GA/AA	186 (79.8)	47 (20.2)		14.3 (1.77–115)	.01
<i>IGF2R</i> L252V						
	CC/CG	237 (84.0)	45 (16.0)	.01*	1.0	
	GG	1 (25.0)	3 (75.0)		23.8 (1.98–287)	.01
<i>IGF2R</i> L2222L						
	CC	198 (86.8)	30 (13.2)	.001	1.0	
	TC/TT	40 (69.0)	18 (31.0)		3.58 (1.59–8.06)	.002
<i>IRS1</i> G972R						
	GG	217 (86.1)	35 (13.9)	<.001	1.0	
	GA/AA	21 (61.8)	12 (36.2)		3.71 (1.43–9.59)	.007
Tumor Stage						
Localized		Advanced				
<i>IGF1R</i> IVS2-70184A>G						
	AA	162 (62.1)	99 (37.9)	<.001	1.0	
	GA	122 (41.4)	173 (58.6)		2.31 (1.57–3.39)	<.001
	GG	33 (28.9)	81 (71.1)		4.23 (2.48–7.19)	<.001
<i>IGF1</i> Ex4+2776G>A						
	GG	178 (58.2)	128 (41.8)	<.001	1.0	
	GA	100 (42.7)	134 (57.3)		1.82 (1.24–2.66)	.002
	AA	23 (37.1)	39 (62.9)		2.62 (1.40–4.89)	.003
<i>IRS2</i> Ex2+750A>G						
	AA/GG	141 (41.7)	197 (58.3)	.004	1.0	

Variable	Genotype	No. (%)	No. (%)	P (χ^2)	OR [†] (95% CI)	P [‡]
	GA	192 (52.5)	174 (47.5)		0.66 (0.47–0.92)	.01
	<i>IRS2</i> IVS1+5687T>C					
	TT	205 (60.8)	132 (39.2)	<.001	1.0	
	CT/CC	121 (35.3)	222 (64.7)		2.51 (1.78–3.55)	<.001
Tumor Resectability						
		Resectable	Unresectable			
	<i>IGF1R</i> IVS2-70184A>G					
	AA	119 (45.6)	142 (54.4)	.002	1.0	
	GA	112 (38)	183 (62)		1.20 (0.82–1.77)	.34
	GG	30 (26.3)	84 (73.7)		2.16 (1.26–3.70)	.005
	<i>IGF1</i> Ex4+2776G>A					
	GG	143 (46.7)	163 (53.3)	.002	1.0	
	GA/AA	102 (34.5)	194 (65.5)		1.63 (1.13–2.36)	.007
	<i>IRS2</i> IVS1+5687T>C					
	TT	164 (48.7)	173 (51.3)	<.001	1.0	
	CT/CC	104 (30.3)	239 (69.7)		1.83 (1.29–2.60)	.001
	<i>IGF1R</i> T766T					
	CC	257 (40.9)	371 (59.1)	.002	1.0	
	CT/TT	18 (23.1)	60 (76.9)		2.48 (1.33–4.63)	.004

OR, odds ratio; CI, confidence interval; PR, partial response; SD, stable disease; PD, progressive disease; CA19-9, carbohydrate antigen 19-9.

* P value from Fisher's exact (2-sided) test.

[†] OR was calculated from logistic regression adjusted sex, race, clinical stage, tumor resection, CA 19-9, biochemical index and performance status when appropriate.