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Genetic and Expression Analysis of MET, MACC1, and HGF in Metastatic Colorectal Cancer: Response to Met Inhibition in Patient Xenografts and Pathologic Correlations

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ABSTRACT

Purpose: We determined the gene copy numbers for MET, for its transcriptional activator MACC1 and for its ligand hepatocyte growth factor (HGF) in liver metastases from colorectal carcinoma (mCRC). We correlated copy numbers with mRNA levels and explored whether gain and/or overexpression of MET and MACC1 predict response to anti-Met therapies. Finally, we assessed whether their genomic or transcriptional deregulation correlates with pathologic and molecular parameters of aggressive disease.

Experimental Design: One hundred three mCRCs were analyzed. Copy numbers and mRNA were determined by quantitative PCR (qPCR). Thirty nine samples were implanted and expanded in NOD (nonobese diabetic)/SCID (severe combined immunodeficient) mice to generate cohorts that were treated with the Met inhibitor JNJ-38877605. *In silico* analysis of MACC1 targets relied on genome-wide mapping of promoter regions and on expression data from two CRC datasets.

Results: No focal, high-grade amplifications of MET, MACC1, or HGF were detected. Chromosome 7 polysomy and gain of the p-arm were observed in 21% and 8% of cases, respectively, and significantly correlated with higher expression of both Met and MACC1. Met inhibition in patient-derived xenografts did not modify tumor growth. Copy number gain and overexpression of MACC1 correlated with unfavorable pathologic features better than overexpression of Met. Bioinformatic analysis of putative MACC1 targets identified elements besides Met, whose overexpression cosegregated with aggressive forms of colorectal cancer.

Conclusions: Experiments in patient-derived xenografts suggest that mCRCs do not rely on Met genomic gain and/or overexpression for growth. On the basis of pathologic correlations and bioinformatic analysis, MACC1 could contribute to CRC progression through mechanisms other than or additional to Met transcriptional upregulation.

INTRODUCTION

With more than 400,000 cases each year, colorectal cancer (CRC) is the third most common malignancy and the second leading cause of cancer deaths in the western world, with an ever increasing global incidence (1, 2). Approximately half of CRC patients—namely, those with localized or only locally advanced disease—can be cured by surgery and multimodal therapy; the other half present at diagnosis with distant (usually liver) metastasis, a setting that portends a dismal survival outcome. Indeed, despite therapeutic advances, the prognosis for patients with unresectable metastatic CRC (mCRC) remains unfavorable, with a median overall survival of 18 to 21 months (3–5).

The fact that the metastatic process is directly linked to patient survival necessitates the search of molecular biomarkers for the early identification of tumors with elevated metastatic propensity. This endeavor is further supported by the notion that, in principle, prognostic biomarkers can also act as predictors of response to targeted treatments (6). A paradigmatic example is provided by the *HER2* gene, whose amplification in mammary tumors foretells aggressive growth and a high risk of relapse following conventional chemotherapy, but in the meantime predicts cancer sensitivity to HER2 inhibitors (7). Therefore, prognostic determinants may become potential therapeutic targets, provided that their expression does not simply correlate with the probability of tumor dissemination but also plays a causative role in the onset of the metastatic phenotype.

The *MET* oncogene encodes for the Met tyrosine kinase receptor for HGF (8). Met is aberrantly activated in a vast spectrum of human cancers due to gene amplification, transcriptional upregulation, point mutations, or ligand autocrine loops (9, 10). Cell lines exhibiting amplification of the *MET* gene respond to Met inactivation with remarkable growth impairment, suggesting that this kind of genetic aberration drives “addiction” to Met activity *in vitro* and may predict effective treatment outcome *in vivo* (11–13). Besides stimulating proliferation, Met also encourages cell scattering, invasion and protection from apoptosis, thereby acting as an adjuvant prometastatic gene for many tumor types (14).

In colorectal cancer, Met is considered important for the metastatic potential to the liver and represents a powerful prognostic indicator for early stage invasion and metastasis: high expression of Met in CRCs associates with development of distant metastases and with shorter metastasis-free survival (15–19). This notion has been recently corroborated by the finding that MACC1, an upstream transcriptional activator of Met, is also an independent prognostic predictor of metastasis formation and metastasis-free survival in mCRCs (19). Finally, the seeding of metastatic cells in the liver leads to a reactive hepatic pathology that is accompanied by a surge of circulating HGF, which might further activate Met by systemic/paracrine mechanisms (20). These observations have prompted the design of clinical trials that are currently testing Met and HGF inhibitors in mCRCs (21).

On the basis on these premises, we decided to carry out a detailed analysis of the genomic status and of the expression levels of MET, MACC1, and HGF in a cohort of 103 consecutive liver metastases from colorectal carcinomas. The aim was 2-fold: (i) to explore whether copy number variations of MET, MACC1, and HGF, as well as their overexpression, can predict response to Met targeted therapies (using mCRC patient-derived xenografts as preclinical readouts of therapeutic efficacy); and (ii) to assess whether their genomic or transcriptional deregulation correlates with pathologic and molecular parameters of aggressive disease.

MATERIALS AND METHODS

Specimen collection and annotation

A total of 103 consecutive tumor samples and matched normal samples were obtained from patients treated by liver metastasectomy at IRCC, Ordine Mauriziano, and San Giovanni Battista Hospitals (Torino, Italy). All patients provided informed consent and samples were procured and the study was conducted under the approval of the Review Boards of the 3 institutions. Clinical and pathologic data were entered and maintained in our prospective database.

Analyte extraction

Nucleic acids were isolated from surgically resected colorectal cancer liver metastases and from matched normal liver tissues, following overnight incubation of the fresh specimens in RNAlater (Ambion), quick freezing at −80°C and mechanical fragmentation. Genomic DNA was isolated using the Blood & Cell Culture DNA Midi Kit (Qiagen). Total RNA was extracted using the miRNeasy Mini Kit (Qiagen) and quality checked by measuring the 28S/18S ribosomal RNA ratio with an Agilent 2100 Bioanalyzer (Agilent Technologies). DNA and RNA concentrations were quantified using a Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific).

Gene copy number and expression analysis

qPCR on genomic DNA and cDNA was carried out using the Power Sybr PCR Master Mix (Applied Biosystems) and the 7900 HT Abiprism Real-Time System (Applied Biosystems). cDNA was produced using the Reverse Transcription System (Promega), according to the manufacturer's instructions. RPS6KC1 was chosen as a reference for gene dosage normalization. Hypoxanthine-guanine phosphoribosyltransferase and the subunit A of the succinate dehydrogenase complex were chosen as references for transcript normalization. The list of primers used for gene copy number and gene expression analyses (Sigma Aldrich) is presented in Supplementary Table S1.

Explant xenograft models

Tumor material not required for histopathologic analysis was collected and placed in medium 199 supplemented with 200 U/mL penicillin, 200 µg/mL streptomycin, and 100 µg/mL levofloxacin. Each sample was cut into 25- to 30-mm^3 pieces in antibiotic-containing medium; some of the pieces were incubated overnight in RNAlater and then frozen at −80°C for molecular analyses; 2 other pieces were coated in Matrigel (BD Biosciences) and implanted in 2 different 4- to 6-week-old female NOD (nonobese diabetic)/SCID (severe combined immunodeficient) mice. After mass formation, the tumors were passaged and expanded for 2 generations until production of a cohort of 12 or 24 mice, depending on the amount of the original material. For each cohort, half of the animals were treated with vehicle and half were dosed with 40 mg/kg/die of the Met small molecule inhibitor JNJ-38877605. Compound concentration in the tumor sites was analyzed by liquid chromatography/mass spectrometry. Tumor size was evaluated once per week by caliper measurements and the approximate volume of the mass was calculated using the formula $4/3\pi \cdot (d/2)^2 . D/2$, where d is the minor tumor axis and D is the major tumor axis. All animal procedures were approved by the Ethical Commission of the Institute for Cancer Research and Treatment (Candiolo, Torino, Italy), and by the Italian Ministry of Health.

Statistics

Data are expressed as mean ± SEM. Comparisons were made using the 2-tailed Student's*t* test. Association between pathologic parameters and MACC1 copy number was carried out by a paired univariate statistical analysis using Fisher's exact test.

In silico analyses

A detailed description of the strategy used to identify MACC1 putative targets can be found in the Supplementary Data.

RESULTS

Copy-number analysis of MET, MACC1, and HGF in liver metastases from colorectal carcinomas

103 consecutive liver metastases from colorectal carcinomas were evaluated for copy number alterations of the MET, MACC1, and HGF genes using quantitative real-time PCR (qPCR) on genomic DNA extracts. The 3 genes all lie along chromosome 7: MET and HGF occupy a subtelomeric and a centromeric position, respectively, on the q-arm, whereas MACC1 is located at a telomeric position on the p-arm (Fig. 1A). EGFR, encoding the epidermal growth factor receptor, was used as an intrachromosomal control to cover a centromeric locus on the p-arm. RPS6KC1 was chosen as a reference for gene dosage normalization: this gene is contained in the subtelomeric region of chromosome 1q, which is typically spared by events of chromosomal instability in colorectal cancer (22). A summary of the clinical characteristics for the study cohort can be found in Table 1 and detailed information is provided in Supplementary Table S2.

With the exception of a 32-fold peak of copy number gain for EGFR in 1 case, we did not detect focal, high-grade amplification of MET, MACC1, HGF, or EGFR in any of the samples analyzed (Supplementary Table S3). Low-level polysomy of chromosome 7 was observed in 22 cases (21%), with an average of 3.3 copies. In 9 cases (7.7%) we could distinguish a specific increase in the ploidy of the p-arm (average, 3.4 copies), with genomic overrepresentation of MACC1 and EGFR and a normal diploid status for MET and HGF. In 3 cases (3%), moderate gain of the p-arm (average, 6.8 copies) appeared to be superimposed to a condition of whole chromosome 7 polysomy (Fig. 1B). In sum, polysomy of the entire chromosome 7 or selective gain of the p-arm appear to be a relatively frequent occurrence in mCRCs; at variance, locus-specific copy number alterations of MET, MACC1, and HGF are likely to be at very low frequencies, if not absent.

Expression analysis of Met and MACC1 in liver metastases from colorectal carcinomas

To test whether the observed copy number alterations of chromosome 7 drive aberrant expression of Met, we integrated the genomic data with transcriptional analyses (Supplementary Table S3): comparison of relative Met transcripts with the ploidy condition of chromosome 7 revealed that higher Met expression associated with chromosome 7 polysomy, consistent with a gene dosage effect. However, gain of MET was not the sole genomic determinant of Met overexpression: the average transcript levels of Met were even more pronounced in those samples that displayed specific gain of the p-arm, where the MACC1 locus—but not the MET locus—resides (Fig. 2A). As expected, expression of MACC1 was prominent in samples with gain of chromosome 7, independent of whether the genetic lesion consisted of whole chromosome polysomy or gain of the p-arm (Fig. 2B).

The observation that Met expression is more elevated in samples in which MACC1 exhibits a genomic gain, irrespective of MET status, indirectly suggests that MACC1 is an upstream regulator of Met levels. Indeed, it has been recently shown that Met is a transcriptional target of MACC1, and Met mRNA expression was found to correlate with that of MACC1 in 60 cases of primary colorectal carcinomas (19). We validated this association also in liver secondary lesions: when the 103 metastases were matched with respect to Met and MACC1 mRNA levels, a direct correlation between Met and MACC1 expression was confirmed (Pearson correlation coefficient = 0.64; Fig. 2C and D). Concurrent direction in the expression of the 2 genes (over or below the median distribution of both) was found in 78 of 103 samples (76%; hypergeometric distribution P =

3.82083E-07; Fig. 2C and D). For control purposes, we also compared the expression levels of MACC1 and HGF: in this case, no correlation was detected (Fig. 2E), further indicating specific coregulation for Met and MACC1.

Response of patient-derived xenografts to Met targeted therapy

It is now well recognized that the major determinant of responsiveness to targeted therapeutics is the presence of a constitutively hyperactive form of the druggable molecule, which usually occurs as a consequence of genetic abnormalities such as point mutations, gene amplification, or chromosomal translocation. Despite the fact that we have been unable to detect a focal, high-grade amplification of the *MET* gene, the finding that Met overexpression tends to correlate with a genetic anomaly—either polysomy of the entire chromosome 7 or gain of the p-arm—supports the notion that targeting Met might have therapeutic value in the context of mCRCs.

To tackle this issue, we subcutaneously implanted mCRC samples from our series in immunocompromised NOD/SCID mice and evaluated the efficacy of systemic inhibition of Met in these patient-derived xenografts. Through 2-step *in vivo* passaging and expansion of the first 39 consecutive specimens that successfully engrafted, we developed 39 cohorts of mice bearing liver metastases; each cohort was generated from a unique patient and consisted of 12 or 24 animals, depending on the amount of the original surgical material (Supplementary Table S3). Consistent with the data obtained in the entire series, this xenografted collection featured 9 cases of chromosome 7 polysomy (23%) and 3 cases of gain of the p-arm (8%; Supplementary Table S3). The distribution of Met expression in the samples implanted is presented in Figure 3A. For each cohort, half of the animals were treated with vehicle and half were dosed with JNJ-38877605, a Met-specific small molecule inhibitor that entered Phase I clinical trials (21, 23–25). We used 2 treatment schedules: in 21 cases, treatment with the Met inhibitor was started the day after tumor implantation (“early treatment”); in 18 cases, the inhibitor was administered when the tumor had reached a volume of approximately 300 to 400 mm^3 (“delayed treatment”; Supplementary Table S3).

Unexpectedly, none of the tumors responded to the treatment, independently of Met expression, chromosome 7 polysomy, or gain of the p-arm. Not only did we not observe cases of regression or stabilization but also we could not even detect any substantial changes in the growth curves following Met inhibition. Analysis of pharmacokinetic disposition in 10 randomly selected animals indicated intratumoral compound concentrations ranging from 6 to 23 μmol/L, all well above the effective dose for inhibition of Met-driven cell proliferation (23–25; Supplementary Fig. S1). Moreover, the same compound at the same dosage could efficiently suppress the growth of established xenografts when Met-addicted cell lines, such as GTL16, were used (26). Therefore, this lack of effect was not due to lack of compound availability or impaired delivery *in vivo*. Representative cohorts produced from samples with high expression of Met are shown inFigure 3B. This absence of response, at least in this experimental setting and with this specific Met inhibitor, raises some concerns about the validity of targeting Met in mCRC and provides further interest for the outcome of the ongoing clinical trials.

Pathologic correlations

The finding that overexpression of Met in mCRCs does not associate with therapeutic responsiveness to Met targeted therapies prompted us to analyze the correlation between the expression levels of Met and MACC1 and some pathologic features (when available) that are indicative of biological aggressiveness, including grading, the number and maximum diameter of hepatic metastases, and vascular dissemination. With the exception of the grading parameter, both Met and MACC1 displayed a trend of preferential expression in aggressive tumors: the 2 molecules appeared to be more expressed in multiple versus single metastases, in larger (>5 cm)

versus smaller (<5 cm) lesions, and in the presence of intravascular metastatic cells. Notably, the differences in the median expression between highly and poorly aggressive cases were always much more pronounced for MACC1 than for Met (Table 2). As far as grading is concerned, MACC1, but not Met, proved to be more expressed in high-grade tumors. When we applied a single parameter statistical analysis, the correlation between the levels of MACC1 and the presence of metastatic emboli reached significance. Further studies of large cohorts of CRC patients with long-term follow-up information using multiparameter statistical models are needed to definitely address this issue; meanwhile, our findings are in favor of a specific association between MACC1 expression levels and unfavorable pathologic characteristics. This association is further supported by the enrichment for MACC1 copy number gain in aggressive tumors (Table 2).

Together, these results indicate that, in the context of liver metastases from colorectal carcinoma, MACC1 outperforms Met in the correlation with pathologic attributes of tumor evolution. This reinforces the notion that Met overexpression is likely insufficient to contribute autonomously to the growth and further progression of hepatic secondary lesions.

In silico identification of putative MACC1 targets

The observation that MACC1 expression correlates better than Met expression with pathologic parameters of aggressive disease is consistent with previous findings: indeed, it has been shown that the prognostic power of MACC1 in predicting metastasis-free survival is higher than that of Met and that the combination of MACC1 and Met expression does not improve the prognosis either for metastasis or for 5-year survival (19), highlighting the stronger prognostic value of MACC1 *per se*. This suggests that transcripts other than Met can be regulated by MACC1, and possibly contribute to the aggressive phenotype associated with high MACC1 levels.

To address this matter, we decided to search for new candidate MACC1 targets through an*in silico* approach based on 2 fundamental assumptions: i) MACC1 targets should display strong transcriptional coregulation with MACC1; ii) as Met is a validated MACC1 target (19,27), the anatomy of the Met promoter can be used as a reference to extrapolate modules likely to mediate MACC1 transcriptional activity in other genes. On these premises, we analyzed whether genes containing the putative promoter consensus(es) were significantly enriched for MACC1-coregulated genes.

In accordance with previous work showing that the integrity of a specific SP1 binding site in the Met promoter is required for Met regulation by MACC1 (26), we initially selected genes only for the presence of an SP1 consensus and tested whether this was sufficient to enrich for transcripts coexpressed with MACC1 (28). Geneset enrichment analysis (GSEA) of a public dataset comprising 372 CRC samples (GSE2109; see Supplementary Methods for details) indicated that the 3,937 transcripts containing an SP1 binding site in their promoter were poorly enriched for MACC1 coregulated genes when compared with all the 13,937 transcripts explored (NES = 1.091; Supplementary Tables S4 and S5). This suggests that other characteristics present in the Met promoter could contribute to determining the specific nature of MACC1 transcriptional function. Indeed, both efficacy and specificity of transcription factors (TF) and coregulators can be modulated by the presence of multiple binding sites for the TF in the target promoter, by the presence of consensus sites for other TFs, and also by the relative position of the multiple consensus sequences within the promoter (29). On the basis of this, we categorized 4 features of the Met promoter that were tested independently or in combination for their ability to identify sets of transcripts enriched for MACC1 coregulation: (i) the presence of 1 or multiple SP1 binding sites; (ii) the presence of the specific SP1 consensus site that is functional in the Met promoter (the “Met-like consensus”); (iii) the presence of an AP2 binding site (which lies in the core region of the Met promoter in proximity of the functional SP1 consensus); and (iv) the relative disposition of SP1 sites and AP2 sites, as observed in the Met promoter (Supplementary Table S4). Enrichment

analyses based on hypergeometric distribution (30) indicated that genes with a promoter featuring the combination of multiple SP1 sites, including the Met-like SP1 consensus, together with 1 AP2 site (hereafter named the SP1multi-METexact-AP2 geneset—SmuMA—, 469 transcripts) displayed the highest and most significant enrichment for MACC1 coregulated transcripts (hypergeometric *P* value = 0.008; Fig. 4A; Supplementary Tables S4 and S6). At variance, the exact topology of the Met promoter did not further improve the performance of the selection, suggesting that this feature is not relevant for MACC1 activity.

The enrichment for MACC1 coregulated transcripts within the SmuMA geneset was independently verified through GSEA analysis (NES = 1.300; Supplementary Table S5), which further highlighted an inner core of 129 transcripts whose expression was strongly correlated with that of MACC1 (Supplementary Table S7). This core of 129 transcripts was considered as the final geneset of MACC1 putative targets (MAput). To validate our selection pipeline, we carried out the GSEA analysis on a completely independent dataset comprising 290 CRC samples (GSE14333; ref. 31). This confirmed a robust and highly significant enrichment for MACC1 coexpressed transcripts within the MAput geneset (NES = 2.267; FDR < 0.001; Fig. 4B; Supplementary Table S7), supporting the notion that this geneset contains a large fraction of potential MACC1 transcriptional targets. Notably, the core of validated putative targets includes Met (Supplementary Table S7), further corroborating the efficacy of our approach in identifying valuable candidates.

Finally, we challenged the list of candidate MACC1 targets for their ability to stratify patients in groups with different clinical outcomes, based on unsupervised k-means clustering (Fig. 4C). Interestingly, the cluster displaying a lower expression of the MAput geneset was characterized by a significantly lower frequency of metastatic events—either synchronous metastases or recurrences in 5 years—(χ^2 *p* value = 0.03). This is consistent with the idea that MACC1 transcriptional targets could contribute to the aggressive phenotype that is associated with high MACC1 expression.

DISCUSSION

Our comprehensive appraisal of MET, MACC1, and HGF copy number variations in mCRCs revealed that high-level, focal amplification of such genes is likely to be a rare, if not unique, occurrence. This is the first evaluation of the genetic status of MACC1 and HGF in this neoplastic setting. In the case of MET, our data confirm on a larger scale a previous report that, using FISH analysis, described a very low incidence (2%) of locus-restricted amplification of the *MET* gene (32). Conversely, this information contradicts other studies that, using semiquantitative techniques such as southern blotting and conventional PCR on genomic DNA, detected MET genetic amplification in a substantial fraction of liver metastases from colorectal carcinomas, with frequencies spanning from 20% to more than 80% (33, 34). We suspect that, at least in some cases, the reported amplification of MET was in fact a wider gain of entire chromosomal regions or, even more plausibly, a complete polysomy of chromosome 7, where the *MET* gene resides. Indeed, when we assessed the genomic content of chromosome 7 by covering telomeric and centromeric loci on both chromosomal arms, we observed frequent polysomy of chromosome 7 (21%), as well as a recurrent and more localized gain of the p-arm (8%).

Chromosome 7 contains not only MET but also MACC1 and HGF. This suggests a more subtle gene dosage effect for Met expression and activity: in the numerous cases of mCRCs with increased ploidy of chromosome 7, Met could be concomitantly hyperactivated by copy number gain, amplified autocriny, and enhanced transcriptional modulation. Consistently, when we integrated gene copy number estimation with expression analysis, we found that those samples exhibiting chromosome 7 polysomy were also characterized by higher expression levels of Met. It is worth noting that expression of Met was even more prominent in tumors that displayed specific

gain of the p-arm, where the MACC1 gene—but not MET—is located. The association between genomic gain of MACC1 and increased expression of Met, irrespective of the presence of Met gains, supports the notion that MACC1 can control Met levels as an upstream regulator of Met transcription. We also anecdotally note that the median expression of Met, MACC1 and HGF appeared to be slightly higher in patients with previous chemotherapy compared with chemonaïve subjects (Table 1 and Supplementary Table S3); this is in coherence with the established notion that the HGF-Met axis is part of a general "stress-and-recovery" response to cytotoxic insults (8, 35).

To evaluate the efficacy of Met inhibition *in vivo*, we developed a patient-derived human mCRC explant model. By combining the use of severely immunocompromised NOD/SCID mice with optimization of the transfer procedures from sample surgical removal to animal implantation, we were able to achieve a high rate of successful engraftments, with 39 of 46 consecutive surgical samples (85%) giving rise to palpable masses. We can therefore reasonably exclude a bias toward selection of more aggressive cases in our xenograft series. This model of tumor direct transfer from humans to mice has several advantages over conventional xenografts with cultured cell lines: one above all, this approach maintains the subject and tumor variability that occurs in the clinic, and therefore it recapitulates some aspects of population-based studies. Unexpectedly, none of the cases responded to Met inhibition, independently of Met expression, chromosome 7 polysomy, or gain of the p-arm. This information has been obtained consistently in large cohorts of mice, with each cohort generated from a single patient's tumor; hence, it is sufficiently solid to provide some preclinical hints.

This model has also some drawbacks. One obvious limitation is that the subcutaneous milieu in which liver metastasis specimens are implanted does not recapitulate the orthotopic hepatic context. Perhaps more importantly, human tumor stroma becomes depleted on serial passages in the animal and substituted by murine components. This prevents analysis of the potential influence of species-specific host parameters on drug sensitivity, irrespective of tumor-autonomous features. Because HGF is mainly produced by mesenchymal cells and mouse HGF binds human Met with only low affinity (36), our experiments did not address whether paracrine HGF may affect tumor responsiveness to Met inhibition. However, the growth of serially passaged patient-derived xenografts (likely expressing mouse HGF) was similar to, or even faster than, that of primary implants (likely expressing human HGF); although circumstantial, this evidence suggests that mCRCs are not dependent on the availability of species-compatible HGF for their accretion and that the absence of human HGF does not interfere with the tumor-autonomous signaling activity of Met. Although we believe that our "negative" findings will provide critical information for future drug and biomarker development in CRC, we also appreciate that ultimate conclusions on the efficacy of Met inhibitors in this tumor setting can be drawn only when results from ongoing clinical trials will become available.

A number of clues suggest that MACC1 could contribute to colorectal tumor progression more efficiently than Met: (i) besides chromosome 7 polysomy, a specific gain of the p-arm containing the MACC1 locus occurs in approximately 8% of mCRC cases, which implies an evolutionary pressure likely related to the acquisition of malignant traits; (ii) MACC1 outperforms Met in the correlation with pathologic parameters of aggressive disease and has better prognostic power in predicting metastasis-free survival (19); and (iii) the expression of MACC1 putative transcriptional targets is higher in CRC cases with a metastatic tendency; hence, MACC1 may coordinate modulation of complex multigene expression patterns involved in tumor aggressiveness.

On the basis of all these considerations, Met copy number gain and/or overexpression are likely insufficient to produce a state of "addiction" in mCRCs, which is congruent with our observation that individual targeting of Met does not affect the growth properties of mCRCs in xenograft experiments. MACC1 might contribute to colon cancer metastasis through mechanisms other than

or additional to selective upregulation of Met. The biological consequences of MACC1 overexpression in mCRCs and the functional validation of candidate MACC1-regulated genes await further investigation.

Disclosure of Potential Conflicts of Interest

T. Perera is a full-time employee of Janssen pharmaceutical companies of Johnson & Johnson. P.M. Comoglio receives research grants from Janssen pharmaceutical companies. The other authors disclosed no potential conflicts of interest.

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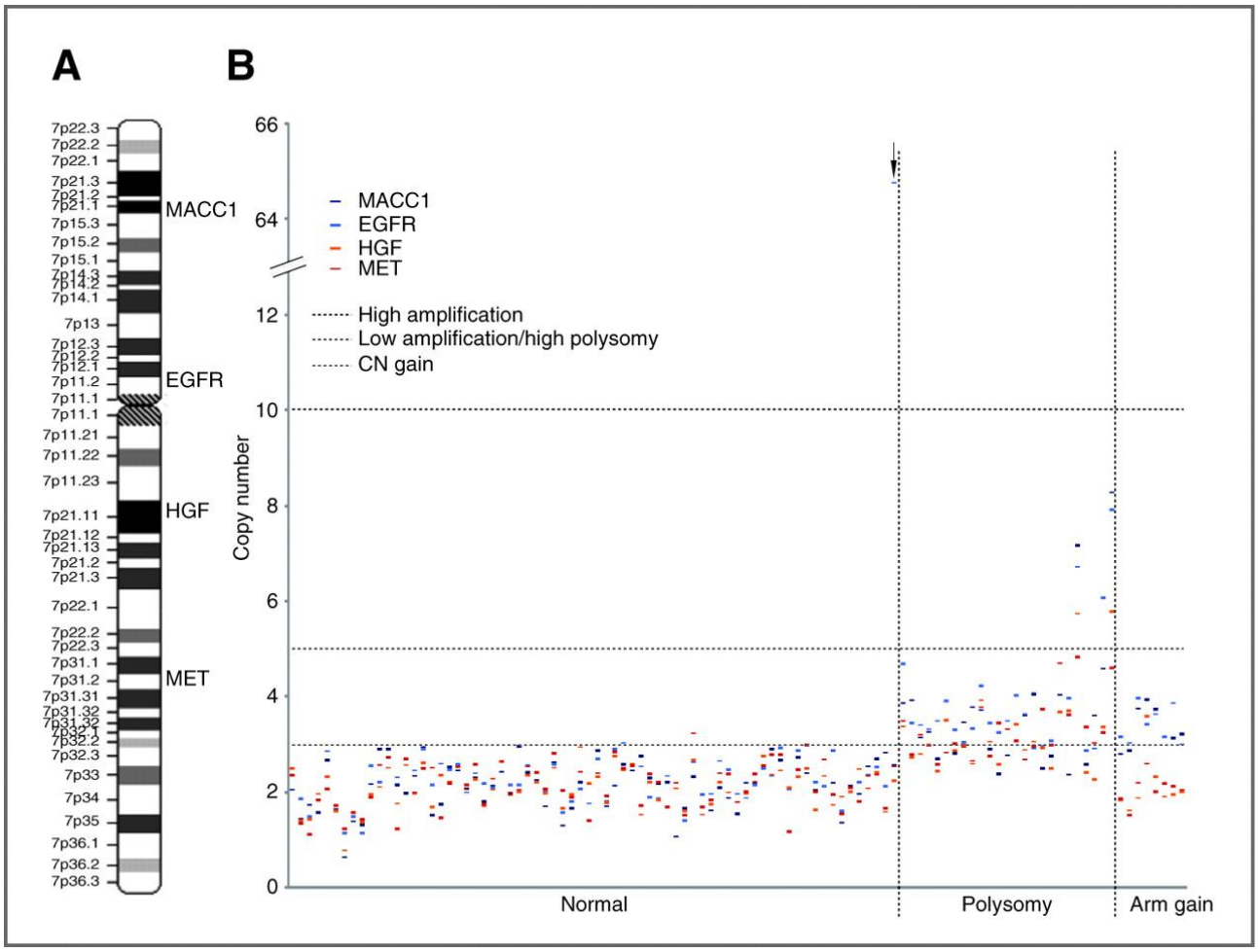

Figure 1.

Copy-number analysis of *MET*, *MACC1*, *HGF*, and *EGFR* genes in liver metastases from colorectal carcinomas. A, locations of the indicated genes along chromosome 7. B, distribution of gene copy numbers for *MET*, *MACC1*, *HGF*, and *EGFR*. Cold colors (blue and pale blue) indicate genes located in the p-arm; warm colors (orange and red) indicate genes located in the q-arm.

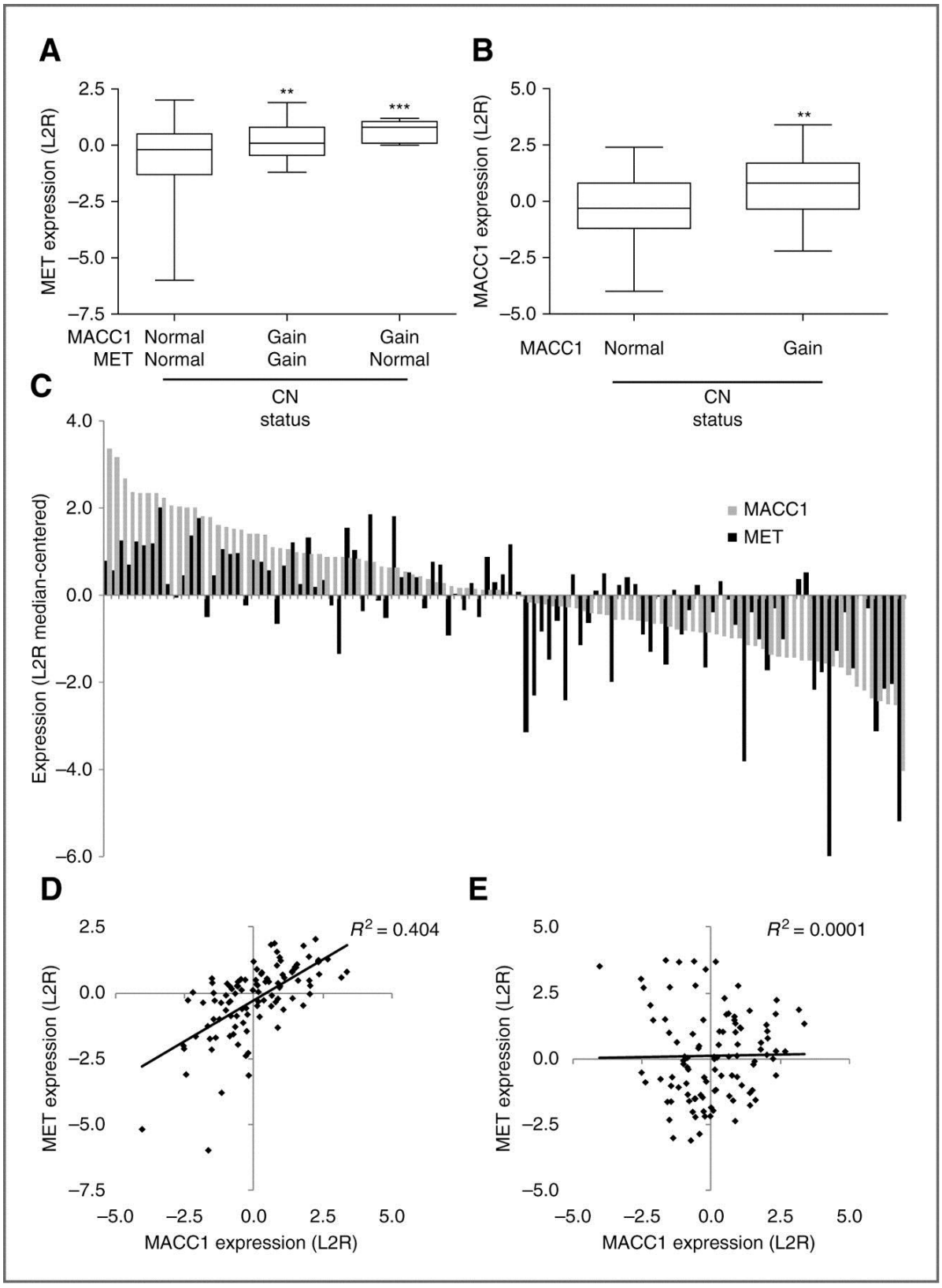

Figure 2.

Expression analysis of Met and MACC1 and correlation with genomic status. Expression of Met (A) and MACC1 (B) with respect to their copy number (CN) status. C, Comparative distribution of the expression levels of Met and MACC1. Two-sample correlation plot of Met and MACC1 expression (D) and MACC1 and HGF expression (E). **, $P < 0.01$; ***, $P < 0.001$.

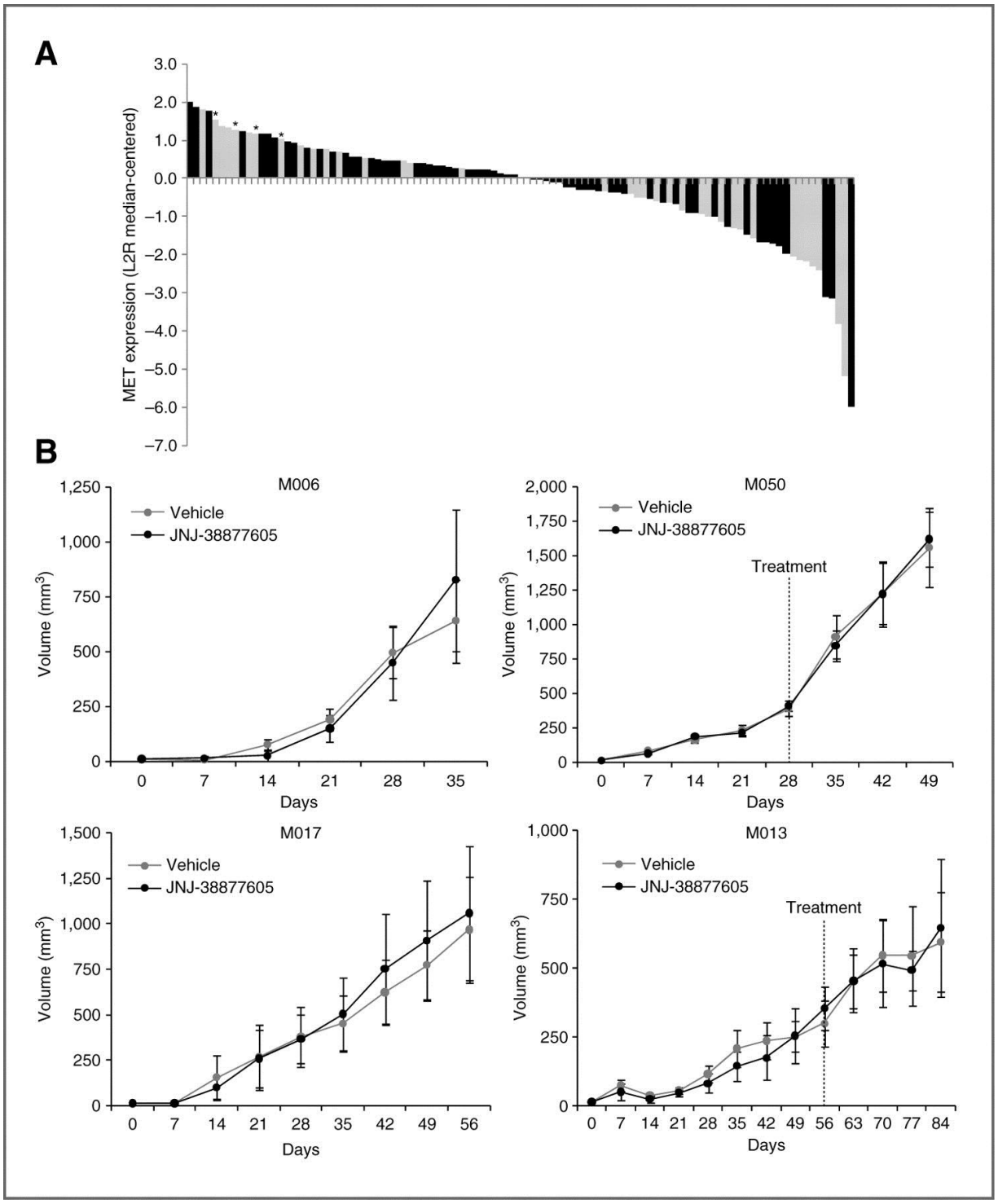

Figure 3.

Lack of response to the Met inhibitor JNJ-38877605 in patient-derived xenografts of liver metastasis from colorectal carcinomas (mCRCs). A, Met expression in mCRCs. Histograms are the same as those reporting Met expression in Figure 2C. Gray histograms denote the samples that were implanted in mice for generation of treatment cohorts. Asterisks indicate the samples for which growth curves are shown in B. B, growth curves of representative patient-derived xenografts (n = 6 for each experimental condition). Left, treatment started the day after tumor implantation; right, treatment started when the tumor had reached an established mass. Data are the means ± SEM (error bars).

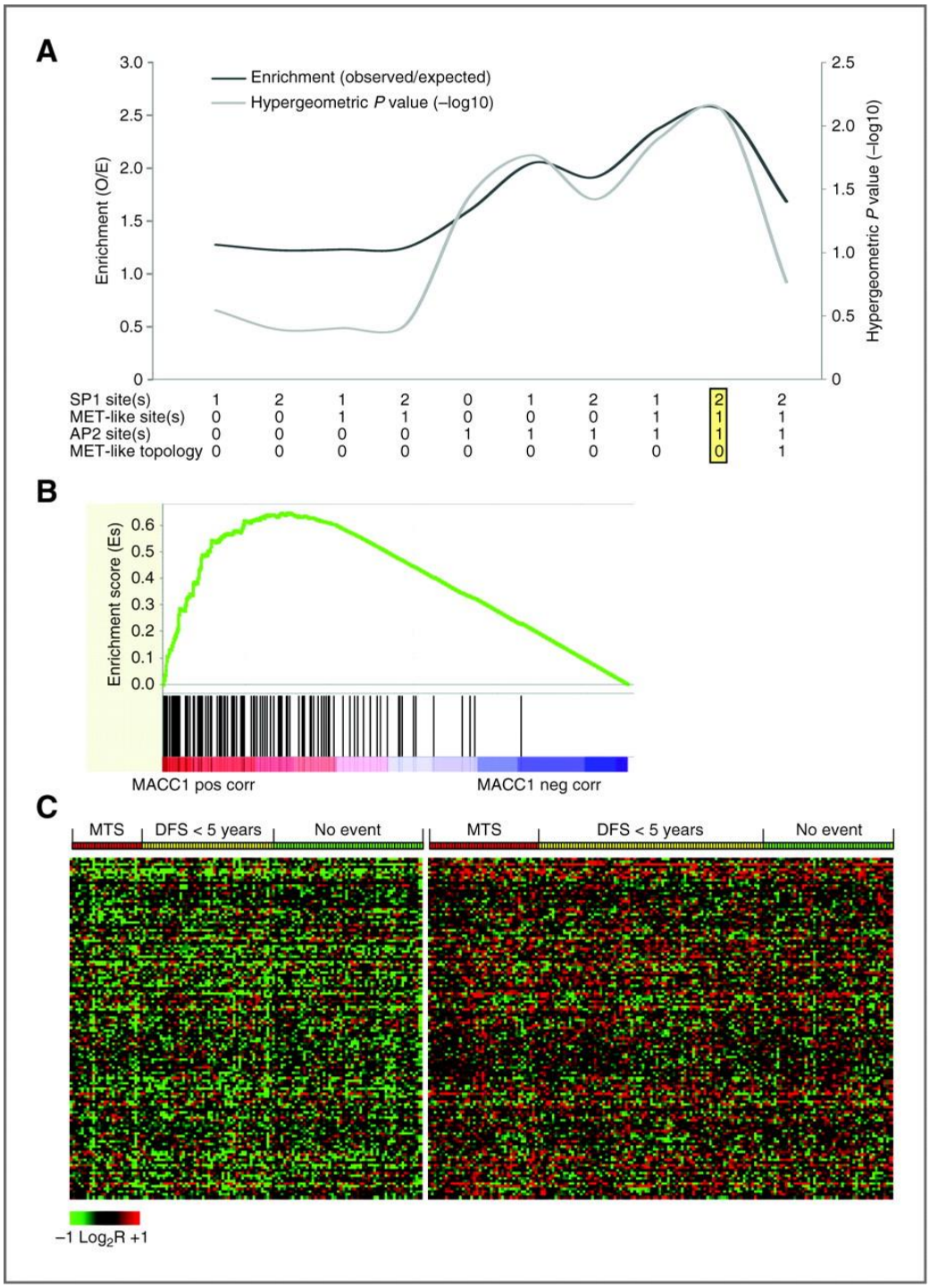

Figure 4.

Identification of new putative MACC1 targets. A, enrichment statistics based on hypergeometric distribution of the Pearson correlation coefficients for the 10 genesets identified by combining Met promoter features. The SmuMA geneset, featuring the highest enrichment and hypergeometric *P* value, is highlighted in yellow. B, GSEA plot of the enrichment analysis for the MAput geneset in the validation dataset, GSE14333. The top portion of the plot shows the running of the enrichment score as the analysis walks down the ranked list; the lower portion of the plot shows where the members of the geneset (black lines) appear in the ranked list of genes (red-blue gradient). C, clustering of the GSE14333 dataset based on MAput expression. Samples are annotated for clinical characteristics as follows: metastatic disease at diagnosis (MTS, red samples); occurrence of metastases within a 5-year follow-up period (disease-free survival, DFS < 5 years, yellow samples); and no metastatic events during follow-up (no event, green samples).

Table 1.

Summary of the clinical characteristics for the study cohort

Sex	
Males	69%
Females	31%
Age, y	
Median	64
Range	46–87
Site of primary	
Colon	78%
Rectum	22%
Diagnosis	
Synchronous	45%
Metachronous	55%
Previous chemotherapy	
Yes	69%
No	31%
Relapse	
First occurrence	86%
Secondary occurrence	14%
Maximum diameter, cm	
Median	3
Range	0.9–20
Number of lesions	
Median	2
Range	1–25
Histologic grade	
1–2	48%
3	52%
Vascular emboli	
No	22%
Yes	78%

Table 2.

Correlations between pathologic parameters and expression of Met, MACC1 and copy number gain (CNG) of MACC1

Parameter		Grading (*n*)			Number (*n*)			Diameter (*n*)			Vascular emboli (*n*)		
		1–2 (37)	3 (34)	*P*value	1 (27)	1 (47)	*P*value	<5cm (56)	>5cm (18)	*P*value	No (10)	Yes (35)	*P*value
Expression (L2R, median)	Met	0.158	−0.023	0.409	−0.296	0.131	0.781	−0.023	0.215	0.220	−0.325	0.332	0.156
	MACC1	−0.258	0.117	0.195	−0.665	0.000	0.119	−0.258	0.764	0.057	−0.693	0.638	0.020
CNG	MACC1	29%	43%	0.216	33%	38%	0.458	32%	44%	0.400	20%	54%	0.077