Multi-Omics Analysis Reveals the Pan-Cancer Landscape of Bone Morphogenetic Proteins

Authors' Contribution:
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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Bone morphogenetic proteins (BMPs) are widely involved in cancer development. However, a wealth of conflicting data raises the question of whether BMPs serve as oncogenes or as cancer suppressors.

Material/Methods: By integrating multi-omics data across cancers, we comprehensively analyzed the genomic and pharmacogenomic landscape of BMP genes across cancers.

Results: Surprisingly, our data indicate that BMPs are globally downregulated in cancers. Further genetics and epigenetics analyses show that this abnormal expression is driven by copy number variations, especially heterozygous amplification. We next assessed the BMP-associated pathways and demonstrated that they suppress cell cycle and estrogen hormone pathways. Bone morphogenetic protein interacts with 58 compounds, and their dysfunction can induce drug sensitivity.

Conclusions: Our results define the landscape of the BMP family at a systems level and open potential therapeutic opportunities for cancer patients.

MeSH Keywords: Bone Morphogenetic Proteins • Epigenomics • Gene Expression Profiling

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Background

Bone morphogenetic proteins (BMPs) were first identified as proteins that are responsible for bone formation. They were first reported to induce the formation of cartilage *in vivo* in 1988 [1] and were believed to be members of the transforming growth factor β (TGF-β) family [1]. BMPs are highly conserved and have existed for over 700 million years [2], which indicates the importance of BMPs in vertebrate physiology. They were later shown to regulate a broad spectrum of biological processes such as cancer development [3]. The BMP family consists of over 10 components [4], some of which are currently in clinical use. Perturbations of BMPs gives rise to a wide range of clinical traits, including tumorigenesis.

In the context of cancer, a few studies have shown that BMPs have widely altered expression in tumor tissues [3]. For example, BMP2 is downregulated in prostate cancer samples and is correlated with cancer progression [5]. In contrast, BMP2 is significantly upregulated in head and neck squamous cell carcinoma [6]. BMP4 was reported by various research groups to have a dual role, acting as an oncogene or a tumor suppressor [7,8]. A wealth of conflicting studies indicated that the same BMP ligand can act differently depending on the cancer type, and it appears that multiple members in the BMP family have distinct functions [3,9]. These seemingly conflicting results raise the critical question of how they are involved and how they influence cancer progression. There is a pressing need to define the role of the BMP family across cancer types.

Here, by using high-throughput sequencing data from a large cohort of patients, we explored the genomic and pharmacogenomic interactions of BMP genes across cancers to assess how BMP family genes are upregulated or downregulated and to determine the cancer hallmark signaling pathways they are associated with. Our work helps define the landscape of the BMP family at the systems level and provides potential therapeutic opportunities for cancer patients.

Material and Methods

Gene expression analysis

We limited our analysis to cancer types with over 10 pairs of matched tumors and normal samples. We conducted paired differential gene expression analysis of tumors and their matched normal samples. The fold change shows the mean gene expression of tumors by dividing the mean gene expression of normal samples. P values were adjusted by FDR. Genes with fold change (FC >2) and significance (FDR >0.05) were used for further analysis.

Survival analysis and cancer subtype analysis

We used sample barcodes to match gene expression and clinical survival data. We used the median RSEM value to divide samples into high and low gene expression groups. Then, we used the R package survival to assess to the survival time and survival status of the 2 groups. We constructed a Cox ratio-hazard model for each gene and plotted the Kaplan-Meier curve using the log-rank test for each gene. We performed ANOVA to find subtype-specific genes.

Single-nucleotide variation analysis

We obtained single-nucleotide variation data from The Cancer Genome Atlas, and we used maftools (*https://bioconductor.org/packages/release/bioc/html/maftools.html*) to analyze the SNV data. SNV percentage refers to the number of mutated samples divided by the number of cancer samples.
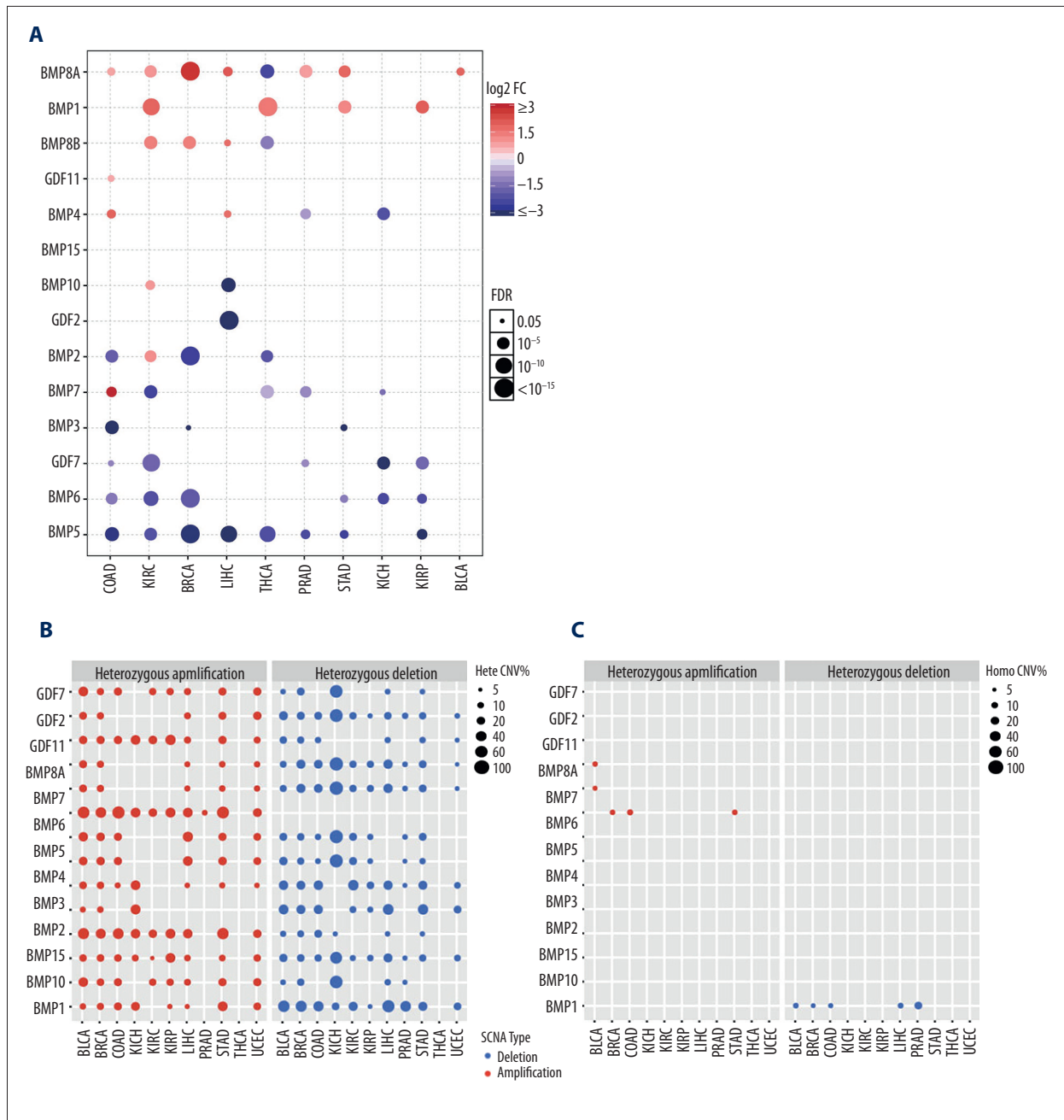
Copy number variation analysis

CNVs are divided into 2 subtypes – heterozygous CNV and homozygous CNV. These 2 subtypes represent only 1 chromosome or 2 chromosomes simultaneously with CNV. Based on the percentage statistics of CNV subtypes, GISTIC (*http://software.broadinstitute.org/cancer/software/genepattern/modules/docs/GISTIC_2.0*) was used to process CNV data, and the calculations used original CNV data and RNA-seq data.

Methylation analysis

We retrieved methylation data from the NCI Genomic Data Commons (*https://gdc.cancer.gov/*). Only cancers paired with normal data were included for analysis. The mRNA expression and methylation data were merged according to their TCGA barcode. We used the methylation of the promoter region of matched genes. We examined the relationship between gene expression and methylation level according to the correlation coefficient of humans. Genes with FDR less than 0.05 were retained.

Pathway activity quantification

RPPA data from The Cancer Proteome Atlas (TCPA) were used to calculate the scores of 10 cancer-related pathways. Reversed Phase Protein Array (RPPA) is a technology with a process similar to Western blot analysis. The RPPA data were centered and the relative protein levels were obtained by normalizing the standard deviations for all samples of each component.

Figure 1. Bone morphogenetic protein family genes are commonly dysregulated in human cancers. (**A**) The CDK gene is downregulated in a wide range of cancers. Copy number variation of the CDK gene includes (**B**) heterozygous amplification and (**C**) homozygous amplification.

Drug response analysis

To analyze the correlation between gene expression and drug sensitivity, we downloaded the region under the drug dose-response curve (AUC) values and the gene expression profiles of all cancer cell lines. Fisher's Z transform was used to normalize the transcription level to the Pearson correlation coefficient of AUCs, with a Bonferroni-corrected 2-tailed distribution with a family error rate of less than 0.025 per tail. The Pearson correlation coefficient of the labeled drug target pair was compared to the same number of correlation coefficients produced by random sampling correlation.
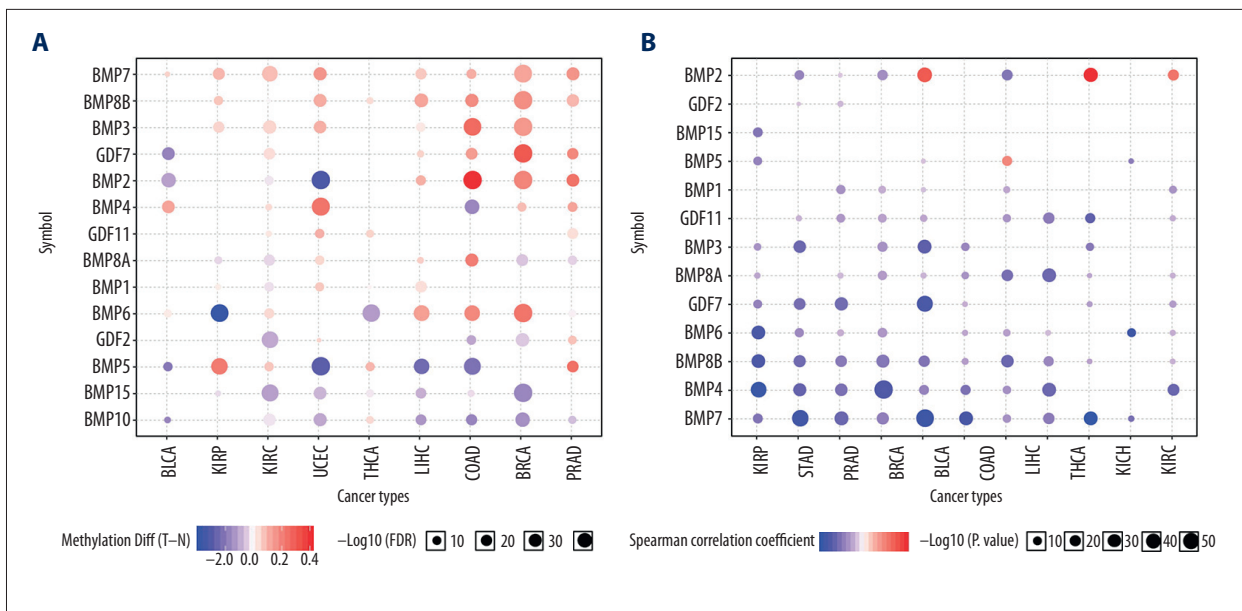
Figure 2. The BMP family is epigenetically demethylated. (**A**) Differential methylation of the BMP genes in human cancers. (**B**) Correlation between methylation and BMP gene expression.

Results

The bone morphogenetic protein family shows globally downregulated expression across human cancers

An unanswered question about BMPs is whether these molecules are downregulated or upregulated [3]. We thus queried the BMP expression landscape across 10 cancers in The Cancer Genome Atlas with matched normal samples. We directly used paired differential gene expression analysis. Surprisingly, we observed that BMP molecules are consistently downregulated across cancers (Figure 1A and Supplementary Figure 1). For example, BMP5 shows globally decreased expression in most cancers. In kidney renal clear cell carcinoma, BMPs show the most alteration, but in bladder urothelial carcinoma only BMP8A is upregulated in tumor samples. To explore the origin of expression alteration, we assessed the copy number variations (CNVs) of the BMP family [10] and surprisingly found that CNVs importantly contribute to expression alteration (Figure 1B, 1C, and Supplementary Figure 2). Specifically, heterozygous amplification, but not heterozygous deletion, is directly correlated with mRNA expression. BMP family dysregulation is also associated with patient survival (Supplementary Figure 3). In summary, the BMP family shows globally downregulated expression across human cancers, in which CNV contributes to expression alteration.

Promoters of the bone morphogenetic protein family are consistently epigenetically methylated

DNA methylation, especially gene promoter methylation, contributes to downregulation of mRNA expression [11]. To determine why BMP molecules are consistently downregulated, we next explored whether methylation is involved in this process. We retrieved the methylome data of pan-cancer patients in The Cancer Genome Atlas and searched their matched RNA-seq data. As expected, most of the BMP family molecules showed an epigenetically silenced trend (Figure 2A). For example, promotors of BMP6 are consistently methylated, and this is in agreement with their expression at the RNA level. Next, we directly computed the spearman Rho estimate to confirm the negative correlation between methylation and gene expression. As expected, methylation level was negatively associated with mRNA expression (Figure 2B). Collectively, our data demonstrate that methylation also contributes to BMP family downregulation.

Mutation also affects the dysfunctional bone morphogenetic protein landscape across cancers

To further understand how BMPs are altered across cancers, we analyzed the mutation profile of BMPs that result in dysfunctional protein function but do not induce expression change. We first assessed the mutation frequency and unexpectedly observed that BMPs, especially BMP1, are frequently mutated (Figure 3A). In 338 analyzed patients, 372 (90.86%) patients showed at least 1 mutation. Particularly in uterine corpus endometrial carcinoma, ~32% samples showed mutated BMP1.
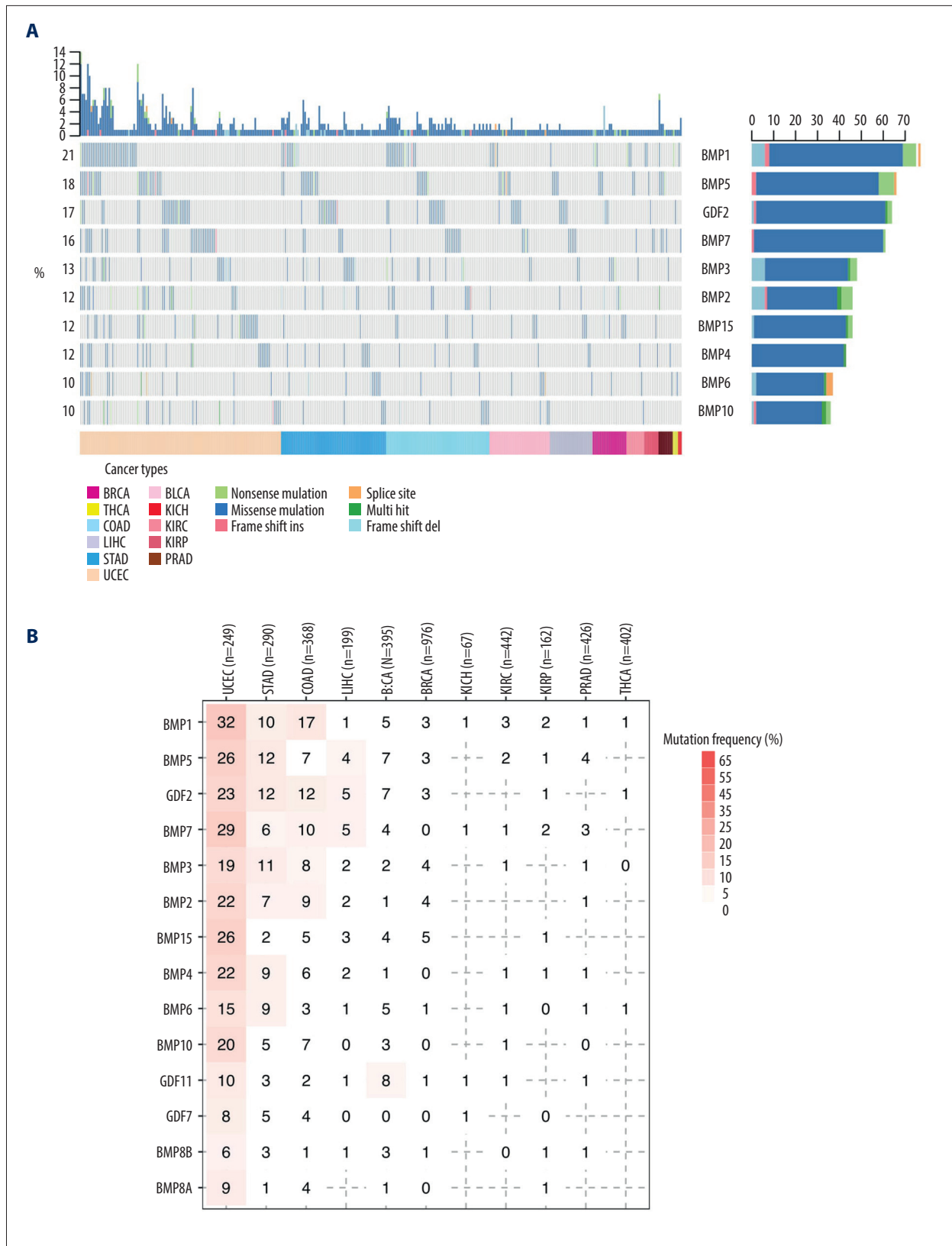
Figure 3. Mutations can also lead to a BMP family that is dysfunctional in cancer. (**A**) Frequency of CDK gene mutation. (**B**) A summary of the variation of each sample.
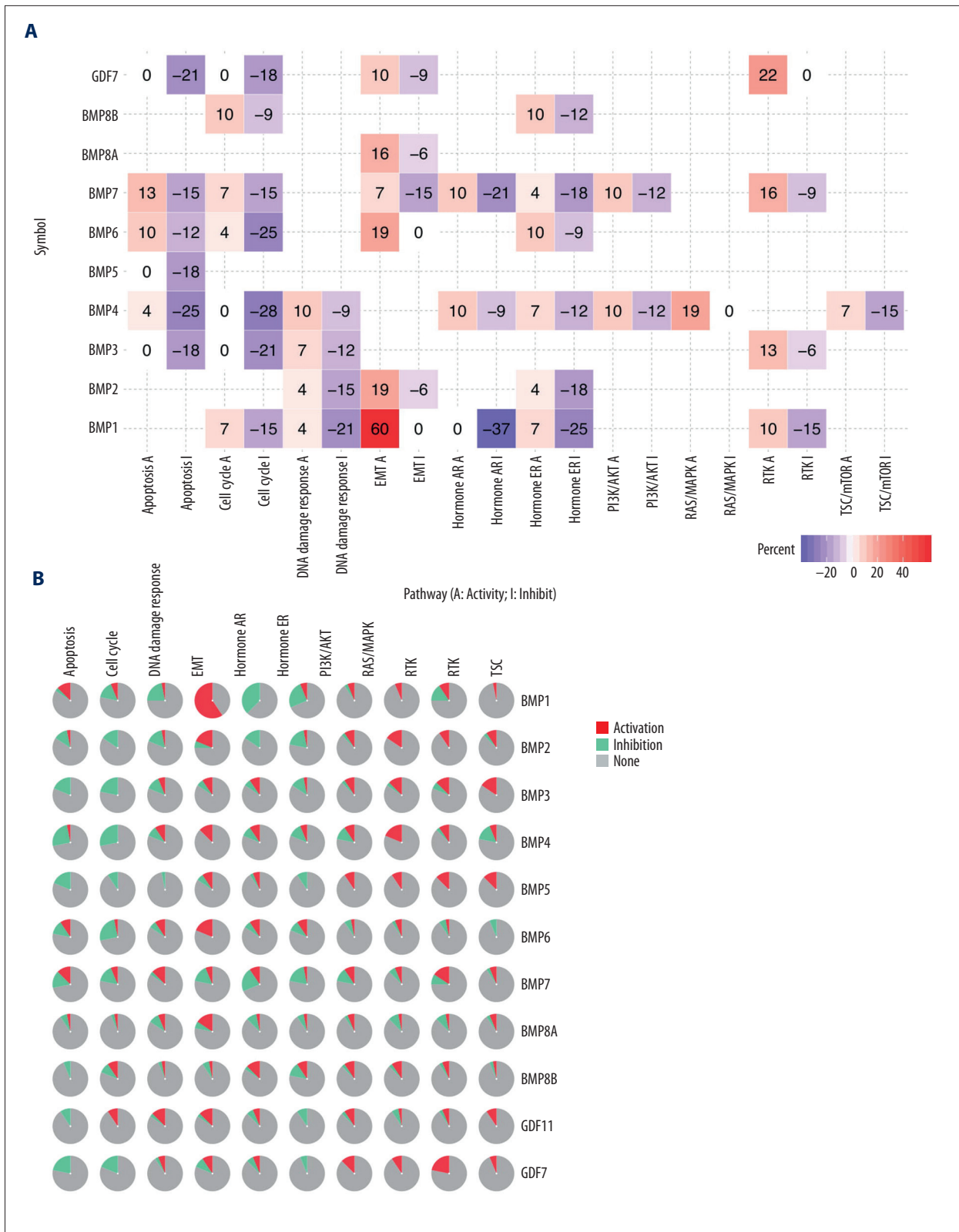
Figure 4. The BMP genes are widely associated with the hallmark cancer pathways. (**A**) A gene heat map with function (inhibition or activation) in at least 5 cancer types. Pathway_a represents the activation of this pathway in a manner similar to pathway_i. (**B**) The pathway activation or inhibition percentage of all BMP genes.

Figure 5. BMP family genes have a broad impact on drug sensitivity in cancer. Dots indicate the relationship between BMP gene expression and drug sensitivity. A positive correlation indicates that the high expression of this gene is resistant.

We next explored which type of mutation contributes to it and interestingly observed that SNP predominates (Figure 3B and Supplementary Figure 4). Most of the mutations are missense and most mutations are C>T. To summarize, besides transcriptional dysfunction, DNA mutation also contributes to abnormal BMPs across cancers.

Bone morphogenetic protein is associated with certain key hallmark cancer pathways

BMPs are globally downregulated in cancers, but it remains unclear how this is associated with certain signaling pathways at the systems level. We thus compared the expression difference between distinct pathway activity groups (activation and inhibition). We selected 10 hallmark cancer pathways and compared them across cancer types (Figure 4A). Interestingly, our results demonstrated that BMP molecules, especially BMP1, are associated with epithelial-mesenchymal transition (EMT). These data are consistent with results of another independent study [12]. Further, we showed that high expression of BMPs is associated with inhibited cell cycle pathway (Figure 4A, 4B). We determined the pathways in the BMP family that are most associated, including EMT, cell cycle, and apoptosis at the systems level.

Bone morphogenetic protein dysfunction induces drug sensitivity

To further investigate the potential effects of BMP genes on drug sensitivity, we examined the correlation between mRNA expression of BMP genes in the GDSC dataset (see Methods). After removing negative molecules, we found that 4 BMP genes were associated with drug sensitivity (Figure 5). Among 481 compounds, we identified 58 compounds that have at least 1 significant correlation with BMP genes. For example, BMP4 shows mostly positive correlations with compounds. These results show the association of BMP genes with drug response.

Discussion

BMPs are involved in a wide range of biological processes such as oncogenesis, but whether they promote or inhibit cancer development remains controversial. A wealth of conflicting studies indicate that the same BMP ligand can act differently depending on the cancer type, and it seems that various members of the BMP family have distinct functions [3,9]. We integrated multi-omics data on thousands of patients and assessed how they are involved and how they affect cancer progression at the systems level.

First, we demonstrated that the bone morphogenetic protein family shows globally downregulated expression across

human cancers. A number of conflicting studies suggested that the same BMP genes act differently depending on the cancer type, and it appears that multiple members of the BMP family have distinct functions [3,9]. For example, BMP2 was previously reported to be downregulated in breast cancer samples [13], which is consistent with our finding. Our results provide more evidence showing that not only BMP2, but also BMP3, BMP5, and BMP6, are downregulated in breast cancer samples. We provide the full map of BMP family expression profile across human tumors.

Second, we showed the reason why BMPs are downregulated. We screened potential expression-drivers from genetics factors to epigenetics factors. We found that copy number variations, especially heterozygous amplification, drive the abnormal expression of BMP genes. At the epigenetics level, most BMP gene promoters are globally methylated. For example, our results demonstrated that BMP6 is methylated in breast cancer samples, which is in line with previous reports [14]. Further, single-nucleotide variants can also contribute to the abnormal function of BMP genes across cancers. Our computational results not only confirm previously reported observations, but also generate new data and hypotheses about abnormal BMP gene expression.

Third, we defined BMP-associated signaling pathways. BMP genes are TGF-β pathway modulators and are widely involved in TGF-β-associated biological processes. To fully define the BMP-associated pathways, we comprehensively analyzed 10 hallmark cancer pathways and quantified their correlation with BMP family expression. Our results show that high expression of BMP triggers cell cycle inhibition across cancers. Other independent research groups also reported similar results, showing that BMP genes are broadly involved in regulating the cell cycle [15,16].

Our study has some limitations. We did not calculate the hazard ratio of each gene in survival analysis due to technical limitations, although it is important for clinicians to interpret those results [17]. We hope future studies will be able to calculate the hazard ratio of each gene. To the best of our knowledge, this study is the first to investigate the effect of BMP genes at the systems level. Further research is needed to confirm our results.

Conclusions

We comprehensively analyzed alterations in BMP genes across multiple human cancers. Our data demonstrate that the BMP family is transcriptionally dysfunctional and shows strong interactions between BMP genes and cancer hallmark pathways, which highlights the importance of BMPs in cancer biology.

Supplementary Data
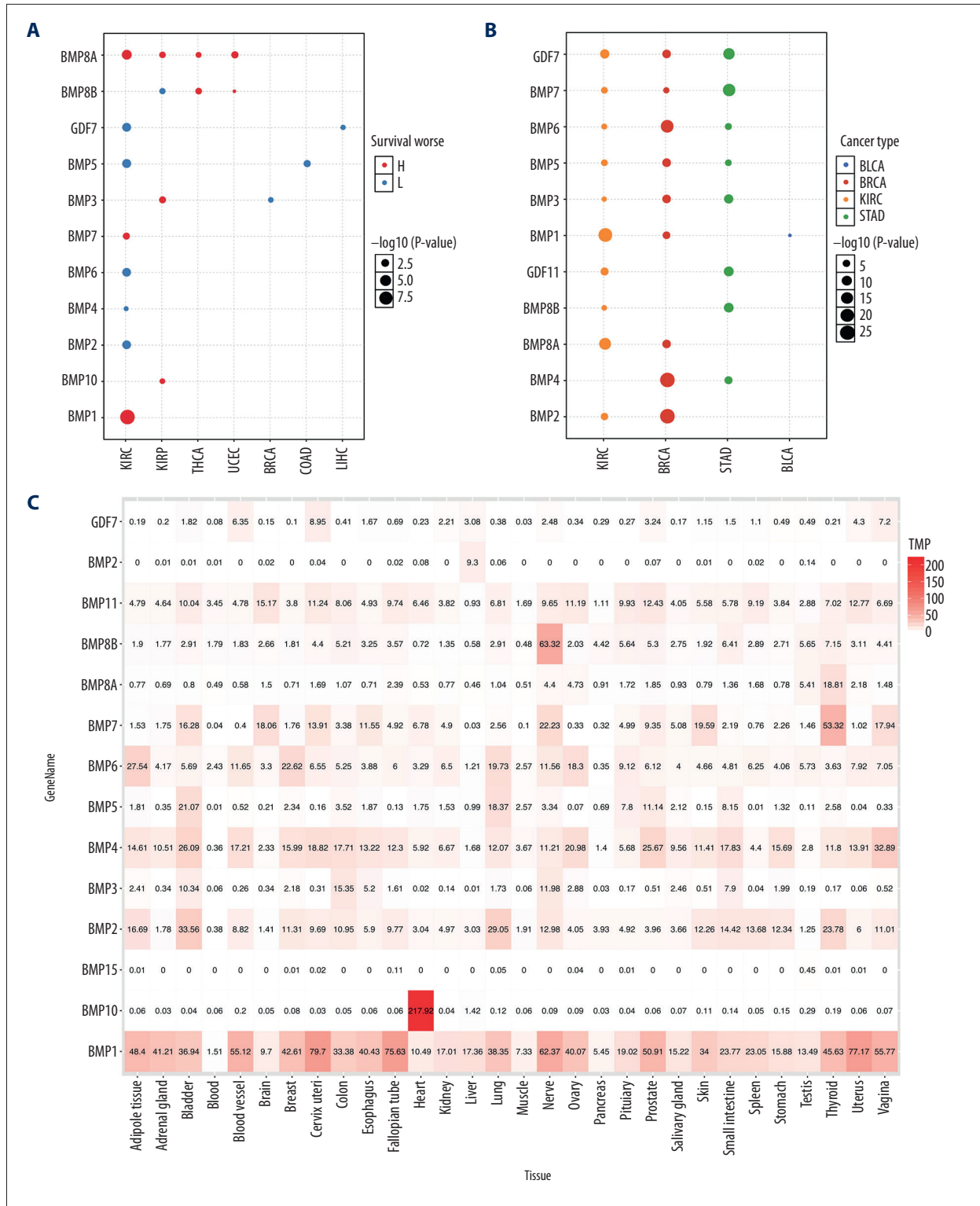
Supplementary Figure 1. The BMP gene is dysfunctional in cancer. (**A**) Prognostic value of the BMP genes. (**B**) Expression of BMP genes is different in cancer subtypes. (**C**) Expression of BMP genes in normal samples.
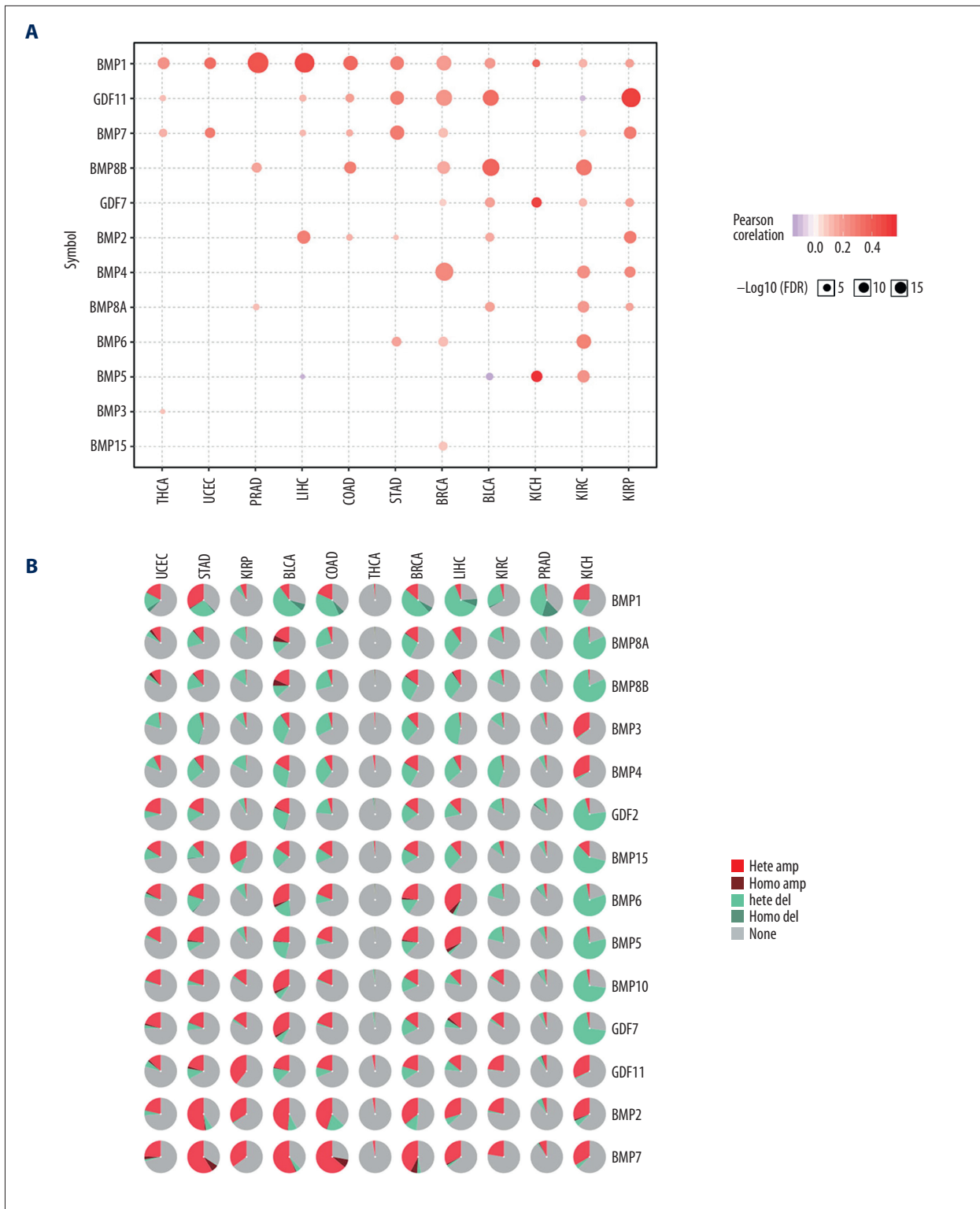
Supplementary Figure 2. Copy number variation affects CDK gene expression. (**A**) Correlation between copy number variation and gene expression. (**B**) Copy number variant subtype of CDK genes in cancer. Hete Amp: heterozygous amplification; Hete Del: heterozygous deletion; Homo Amp: homozygous amplification; Homo Del: homozygous deletion; no: no CNV.

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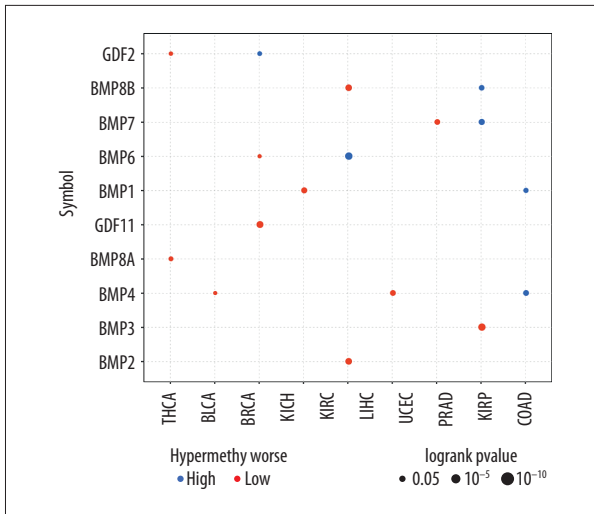
Supplementary Figure 3. The prognostic value of CDK gene methylation in different cancer types.

Supplementary Figure 4. A review of CDK gene variants in human cancers. (**A**) variant type, (**B**) variant classification, (**C**) single-nucleotide variation, (**D**) variation per sample, (**E**) mutation classification, (**F**) mutation of CDK gene between cancer types frequency.

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