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ORIGINAL RESEARCH

A transcriptomic insight into the impacts of mast cells in lung, breast, and colon cancers

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ABSTRACT

To date, the exact impact of mast cells in tumor microenvironment is still controversial because of inconsistency in observations regarding the relationship between mast cell infiltrates and cancer development and prognosis. The discrepancies in previous studies have motivated us to examine the roles of mast cells in cancer pathology from different perspectives. Here, we investigated the impact of mast cells on transcriptomic profiles in the tissue microenvironment. Mice carrying the *W-sh* mutation in *c-kit* ($Kit^{W\text{-}sh}$) are deficient in mast cell production and were used to assess the influence of mast cells on gene expression. By examining the transcriptomic profile among wild-type mice, $Kit^{W\text{-}sh}$ mice, and $Kit^{W\text{-}sh}$ mice with mast cell engraftment, we identified a list of "mast cell–dependent genes," which are enriched for cancer-related pathways. Utilizing whole-genome gene expression data from both mouse models and human cancer patients, we demonstrated that the expression profile of the mast cell–dependent genes differs between tumor and normal tissues from lung, breast, and colon, respectively. Mast cell infiltration is potentially increased in tumors compared with normal tissues, suggesting that mast cells might participate in tumor development. Accordingly, a prognostic molecular signature was developed based on the mast cell–dependent genes, which predicted recurrence-free survival for human patients with lung, breast, and colon cancers, respectively. Our study provides a novel transcriptomic insight into the impact of mast cells in the tumor microenvironment, though further experimental investigation is needed to validate the exact role of individual mast cell–dependent genes in different cancers.

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Introduction

Mast cells are a type of white blood cell derived from bone-marrow haematopoietic progenitors. Immature mast cells circulate in blood until they migrate from vascular to peripheral tissues, where they reside close to blood vessels, nerves, and mucosal surfaces[1] and mature with the help of stem-cell factor and other cytokines secreted by endothelial cells and fibroblasts.[2] Mast cells are usually thought to be deeply involved in inflammatory processes. Once activated, mast cells can rapidly react to xenobiotics by either secreting or releasing mediators from their characteristic granules into the local microenvironment.[3] Disorders of mast cell–activation lead to several immune diseases, such as asthma, eczema, itch, and allergic rhinitis.[4]

Mast cells may be important participants in regulating the tumor microenvironment. Firstly, mast cells are implicated in tumor angiogenesis.[5] Angiogenesis is critical to tumor development. Enhanced vascular permeability and abnormal blood vessel development are often observed in tumors.[6] Mast cells can facilitate tumor angiogenesis by secreting heparin-like molecules, angiogenesis factors (e.g., IL-8),[7,8] and growth factors (e.g., VEGF).[8-10] Decreased tumor angiogenesis has been observed in mast cell–deficient mice.[11] Secondly, mast cells help tumor invasiveness. Several proteases released by mast cells, such as MMP-9,[12,13] and the serine proteases chymase and tryptase,[14] degrade components of the extracellular matrix and thus facilitate tumor invasiveness.[6] Thirdly, mast cells may directly or indirectly interact with immunosuppressive and inflammatory cells in the tumor microenvironment, such as myeloid-derived suppressor cells, tumor-associated macrophages, and regulatory T-cells, to affect immunologic tolerance.[1,15]

Even though there is mounting evidence to indicate mast cell involvement in tumorigenesis, the exact impact of mast cells in the tumor microenvironment is still controversial.[1,6] Particularly, there are several inconsistent observations regarding the relationship between mast cell infiltrates and human cancer development and prognosis. Here, we briefly review the discrepancies in previous studies regarding lung, breast, and colon cancers. For lung cancer, Imada et al. reported that the number of mast cells was positively correlated with angiogenesis and poor outcome in stage I lung adenocarcinoma,[10] which

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was largely mirrored by the study conducted by Takanami et al.,[16] However, Tomita et al. showed in a lung cancer study that the number of mast cells was significantly correlated with a favorable clinical outcome.[17] The latter finding is consistent with the study by Welsh et al.,[18] in which the authors claimed that mast cell–mediated invasion of tumor islets confers a survival advantage in lung cancer.[18] For breast cancer, several studies have linked mast cells to a poor clinical outcome.[19,20] However, a tissue microarray study containing 4,444 cases pointed out that stromal mast cell infiltration in invasive breast cancer is an independent marker of favorable prognosis,[21] which is consistent with the observation of a significant increase in the number of mast cells in tumors from high hormone–receptive cancer cases compared with minimum hormone–receptive cancers.[22] Similar contradictory findings also exist in colon cancer. Mast cell number was positively correlated with microvessel density and associated with a poor prognosis in colon cancer.[23-25] These findings are apparently inconsistent with an earlier observation by Nielsen et al.,[26] in which of 584 colon cancer patients a greater number of tryptase$^+$ mast cells in a tumor specimen correlated significantly with better clinical outcomes.[26]

The discrepancies in these previous studies motivated us to look into the relationship between mast cells and cancer pathology from different perspectives. In this study, we investigated the impact of mast cells on transcriptomic profiles in the tissue microenvironment. Mast cell–deficient *c-kit* mutant rodents, C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ (*Kit*$^{W\text{-}sh}$) mice,[27] were used to assess the influence of mast cells on gene expression of tissue microenvironment. By examining the transcriptomic profile among wild-type (WT) mice, *Kit*$^{W\text{-}sh}$ mice, and *Kit*$^{W\text{-}sh}$ mice engrafted with mast cells derived from WT mice (*Kit*$^{W\text{-}sh+}$MC), we identified a list of "mast cell–dependent genes." Gene ontology analysis indicates that the mast cell–dependent genes are enriched in cancer-related pathways. Utilizing whole-genome gene expression data from mouse models and human cancer patients, we demonstrated that the expression profile of the mast cell–dependent genes differentiates between tumor and normal tissues from lung, breast, and colon, respectively. Accordingly, a prognostic molecular signature was developed based on the mast cell–dependent genes. This signature successfully predicted recurrence-free survival for human patients with lung, breast, and colon cancers in a manner independent of standard clinical and pathological prognostic factors.

Results

Mast cell–dependent genes in mice

To assess the influence of mast cell on gene expression, we compared the gene expression pattern in 3 mouse groups: WT, *Kit*$^{W\text{-}sh}$, and *Kit*$^{W\text{-}sh+}$MC mice. We investigated a transcriptomic data set obtained from the Gene Expression Omnibus (GEO)[28] database (GEO accession: GSE27066),[29] which contains whole-genome gene expression data of WT, *Kit*$^{W\text{-}sh}$, and *Kit*$^{W\text{-}sh+}$MC mouse lung tissues. Gene expression fold changes were computed between *Kit*$^{W\text{-}sh}$ and WT mice (expression in *Kit*$^{W\text{-}sh}$ mice divided by that in WT mice) and between *Kit*$^{W\text{-}sh+}$MC and *Kit*$^{W\text{-}sh}$ mice (expression in *Kit*$^{W\text{-}sh+}$MC mice divided by that in *Kit*$^{W\text{-}sh}$ mice), respectively. A significant negative correlation (Spearman's rank correlation test: $\rho = -0.413$ and $P < 10^{-10}$) was observed between the 2 sets of fold changes (Fig. 1A), which suggests that the deregulation caused by mast cell deficiency could be remarkably recovered by mast cell engraftment. At the specified significance level of false discovery rate <5% and fold change >1.5 (see Methods for details), the expression of 862 genes was downregulated in *Kit*$^{W\text{-}sh}$ mice compared with that in WT mice but upregulated in *Kit*$^{W\text{-}sh+}$MC mice compared with that in *Kit*$^{W\text{-}sh}$ mice, whereas 448 genes were upregulated in *Kit*$^{W\text{-}sh}$ mice compared with that in WT mice but downregulated in *Kit*$^{W\text{-}sh+}$MC mice compared with that in *Kit*$^{W\text{-}sh}$ mice (Fig. 1A). Because the expression pattern of all these deregulated genes showed a largely mast cell–dependent manner, we deemed these genes "mast cell–dependent genes." The genes that were downregulated in mast cell–deficient mice but recovered by mast cell engraftment were deemed mast cell–positive (MC$^+$) genes (Fig. 1B and Supplementary Table S1) whereas the genes that were upregulated in mast cell–deficient mice but restored after mast cell engraftment were considered as mast cell–negative (MC$^-$) genes (Fig. 1B and Supplementary Table S2). We next searched the enriched Kyoto Encyclopedia of Genes and Genomes (KEGG)[30] physiologic pathways among the mast cell–dependent genes. Intriguingly, we found that the top 2 KEGG terms associated with the mast cell–dependent genes were "Pathways in cancer" and "Prostate cancer" (Fig. 1C), which support a significant role for mast cells in cancer pathology. To more precisely understand the biologic processes associated with the mast cell–dependent genes, we further performed pathway/ontology analysis for the MC$^+$ and MC$^-$ genes separately from 3 tumor progression-related aspects: i) immunosuppression,[31-33] ii) apoptosis,[34] and iii) angiogenesis,[35,36] in which mast cells were thought to be implicated. Firstly, we found that the KEGG terms, "T cell receptor signaling pathway" and "Natural killer cell mediated cytotoxicity," were significantly enriched by the MC$^-$ genes but not the MC$^+$ genes (Supplementary Fig. S1A), which suggests that increased mast cell infiltration potentially augments the suppression of T cells and natural killer cells in tumor microenvironment.[31,32] Secondly, we found that the MC$^-$ genes, but not the MC$^+$ genes, were significantly associated with the Gene Ontology (GO)[37] term "Positive regulation of apoptotic process," while the GO term "Negative regulation of apoptotic process" was significantly enriched by the MC$^+$ genes instead of the MC$^-$ genes (Supplementary Fig. S1B), which suggests a potential anti-apoptotic role of mast cells in tumor microenvironment.[34] Thirdly, we found that both the MC$^+$ and MC$^-$ genes were significantly associated with the GO term "Angiogenesis" with a weaker significance level for the MC$^-$ genes, while the GO term "Blood vessel remodeling" was only significantly enriched by the MC$^+$ genes but not the MC$^-$ genes (Supplementary Fig. S1C), which suggests a pro-angiogenic role of mast cells in tumor tissue.[35] These observations further suggest the intrinsic feature of the mast cell–dependent genes regarding immunosuppression, apoptosis, and angiogenesis in tumor microenvironment.

To determine to what extent the mast cell–dependent genes are involved in cancer pathology, we investigated the transcriptomic data in mouse lung (GEO accession: GSE31013),[38] breast

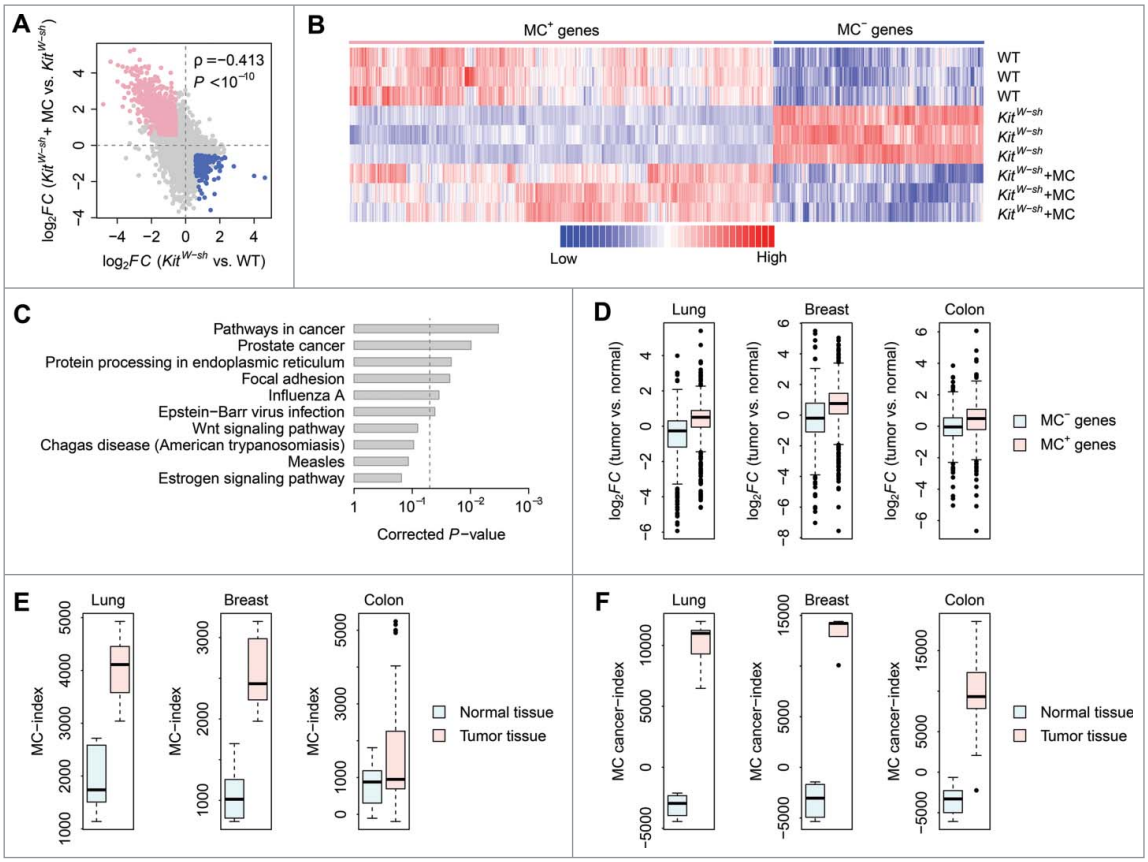

Figure 1. The mast cell–dependent mouse genes. (A) Correlation in $\log_2$-transformed gene expression fold change ($\log_2FC$) between $Kit^{W\text{-}sh}$ and WT mice (X-axis) and between $Kit^{W\text{-}sh+}$MC and $Kit^{W\text{-}sh}$ mice (Y-axis). Each dot stands for a gene. The $\log_2FC$ between $Kit^{W\text{-}sh}$ and WT mice is negatively correlated with the $\log_2FC$ between $Kit^{W\text{-}sh+}$MC and $Kit^{W\text{-}sh}$ mice. Only the genes differentially expressed between $Kit^{W\text{-}sh}$ and WT mice and between $Kit^{W\text{-}sh+}$MC and $Kit^{W\text{-}sh}$ mice in opposite direction were considered as mast cell–dependent genes. The pink dots denote the genes downregulated in mast cell–deficient mice but recovered after mast cell engraftment (MC^+ genes). The blue dots represent the genes upregulated in mast cell–deficient mice but recovered after mast cell engraftment (MC^- genes). (B) Gene expression heatmap of the MC^+ and MC^- genes. Each row in the heatmap denotes one mouse while each column denotes one gene. Red represents relatively increased gene expression whereas blue represents downregulation. (C) The top 10 KEGG pathways associated with the mast cell–dependent genes. The *P*-values were computed by Fisher's exact test and corrected by the Benjamini-Hochberg procedure. The vertical dash-line denotes the significance level of $\alpha = 0.05$. (D) Gene expression fold change of the MC^+ and MC^- genes between mouse tumor and normal tissues. The expression pattern of both the MC^+ and MC^- genes in mouse lung, breast, and colon tumors was compared with normal lung, breast, and colon tissues, respectively. Y-axis denotes the $\log_2FC$ between tumor and normal tissues. (E) Comparison of MC-index between mouse tumor and normal tissues. (F) Comparison of MC cancer-index between mouse tumor and normal tissues.

(GEO accession: GSE21444),[39] and colon (GEO accession: GSE50794)[40] tumors, respectively. Gene expression fold change in mouse lung, breast, and colon tumors were calculated over normal lung, breast, and colon tissues from control mice, respectively. Basically, we found that the $\log_2$-transformed gene expression fold change ($\log_2FC$) of the MC^+ genes was significantly higher than that of the MC^- genes (t-test: $P < 10^{-10}$ for lung and breast; $P = 1.2 \times 10^{-7}$ for colon) (Fig. 1D). One-sample t-test indicates that the $\log_2FC$ of the MC^+ genes is statistically larger than zero ($P < 10^{-10}$) in mouse lung, breast, and colon tumors, respectively (Fig. 1D). On the contrary, the $\log_2FC$ of the MC^- genes is statistically less than zero in lung and breast tumors, but not in colon tumor (one-sample t-test: $P < 10^{-10}$ for lung and breast; $P = 2.2 \times 10^{-1}$ for colon) (Fig. 1D). Taken together, these results suggest that the MC^+ genes, as compared with the MC^- genes, are more likely to be overexpressed in mouse tumors, whereas the MC^- genes, as compared with MC^+ genes, show a higher chance to be downregulated in tumors.

We hypothesized that the mast cell–dependent tissue microenvironment could be delineated from expression deregulation profiles of the mast cell-dependent genes. Here, we developed a novel methodology to compute a mast cell index (MC-index) for individual tissue samples, based on the rank-weighted gene expression information of the MC^+ and MC^- genes (see Materials and Methods for details). We speculated that the MC-index could be used as a proxy of the impact of mast cells on shaping tissue microenvironment. Fig. 1E provides a comparison of MC-index between tumor and normal tissues from mouse lung, breast, and colon, respectively. The MC-index of tumor tissues was significantly higher than that of normal controls (t-test: $P = 2.1 \times 10^{-4}$ for lung; $P = 1.2 \times 10^{-3}$ for breast; $P = 2.4 \times 10^{-3}$ for colon), which suggests an active role for mast cells in tumor development. To more precisely assess the impact of mast cells on cancer pathology and tumor microenvironment, we made some modifications to the algorithm for computing MC-index: at the specified significance level of false discovery rate <5% and fold change >1.5, only the MC^+ genes commonly upregulated and the MC^- genes commonly downregulated in lung, breast, and colon tumors were considered. We deemed these genes "mast cell–dependent cancer genes." A mast-cell cancer index (MC cancer-index) was calculated for

individual tissue samples using the rank-weighted gene expression data of the mast cell-dependent cancer genes (see Materials and Methods for details). Fig. 1F indicates that the MC cancer-index of tumor tissues was significantly higher than that of normal controls (t-test: $P = 1.6 \times 10^{-6}$ for lung; $P = 5.5 \times 10^{-7}$ for breast; $P < 10^{-10}$ for colon). In comparison with MC-index, the difference in MC cancer-index between tumor and normal tissues was even larger (Fig. 1F).

MC- and MC cancer-indices of human cancer patients

To assess the depth of involvement of mast cells in human cancers, we mapped the mast cell-dependent mouse genes to their distinct human orthologs. Next, we investigated the expression pattern of the mast cell-dependent human genes in 6 independent cancer cohorts: 2 lung cancer cohorts from Spain (Lung-ESP, GEO accession: GSE18842)[41] and Taiwan (Lung-TWN, GEO accession: GSE19804),[42] respectively; 2 breast cancer cohorts from the United States (Breast-USA1, GEO accession: GSE70947) and Malaysia (Breast-MYS, GEO accession: GSE15852),[43] respectively; and 2 colon cancer cohorts from Japan (Colon-JPN, GEO accession: GSE22598)[44] and Singapore (Colon-SGP, GEO accession: GSE10950),[45] respectively. We chose these data sets based on the availability of paired transcriptomic data from both tumor and normal tissues. In total, paired tumor and normal tissues from 44 lung cancer patients from the Lung-ESP cohort, 60 lung cancer patients from the Lung-TWN cohort, 148 breast cancer patients from the Breast-USA1 cohort, 43 breast cancer patients from the Breast-MYS cohort, 17 colon cancer patients from the Colon-JPN cohort, and 24 colon cancer patients from the Colon-SGP cohort were investigated. Fig. 2A indicates that the MC-index of the tumor tissues was significantly higher than that of the matched normal tissues in all the 6 human cancer cohorts (paired t-test: $P = 4.3 \times 10^{-2}$ for Lung-ESP; $P = 1.1 \times 10^{-2}$ for Lung-TWN; $P < 10^{-10}$ for Breast-USA1; $P = 1.5 \times 10^{-8}$ for Breast-MYS; $P = 7.5 \times 10^{-4}$ for Colon-JPN; $P = 4.3 \times 10^{-2}$ for Colon-SGP). An even more significant difference between tumor and normal tissues was observed for the MC cancer-index in all these cohorts (paired t-test: $P < 10^{-10}$ for Lung-ESP; $P < 10^{-10}$ for Lung-TWN; $P < 10^{-10}$ for Breast-USA1; $P = 5.6 \times 10^{-8}$ for Breast-MYS; $P = 1.3 \times 10^{-7}$ for Colon-JPN; $P < 10^{-10}$ for Colon-SGP) (Fig. 2B). All these results were highly consistent with our observations in mouse tumors, which suggests the similar significant impact of mast cells on human cancer development.

Because the MC cancer-index was computed based on the mast cell dependent genes commonly deregulated in mouse lung, breast, and colon tumors, we further tested whether this computational model is applicable to other cancer types. Three human cancer cohorts were considered here: one liver cancer cohort from the United States (GEO accession: GSE14520),[46] one prostate cancer cohort from the United States (GEO accession: GSE32448),[47] and one thyroid cancer cohort from Belgium (GEO accession: GSE33630).[48] In total, paired tumor and normal tissues from 214 liver cancer patients, 40 prostate cancer patients, and 44 thyroid cancer patients were investigated. Supplementary Fig. S2 indicates that the MC cancer-index of the tumor tissues was significantly higher than that of the matched normal tissues in liver, prostate, and thyroid cancers (paired t-test: $P < 10^{-10}$ for liver; $P = 3.5 \times 10^{-4}$ for prostate; $P < 10^{-10}$ for thyroid), which suggests the predictive power of MC cancer-index in these cancer types, resonating with our observations in lung, breast, and colon cancers.

Prognostic power of mast cell–dependent cancer genes

We hypothesized that the mast cell–dependent cancer genes would be predictive of cancer outcome and consequently designated these genes as the Mast Cell–Dependent Cancer

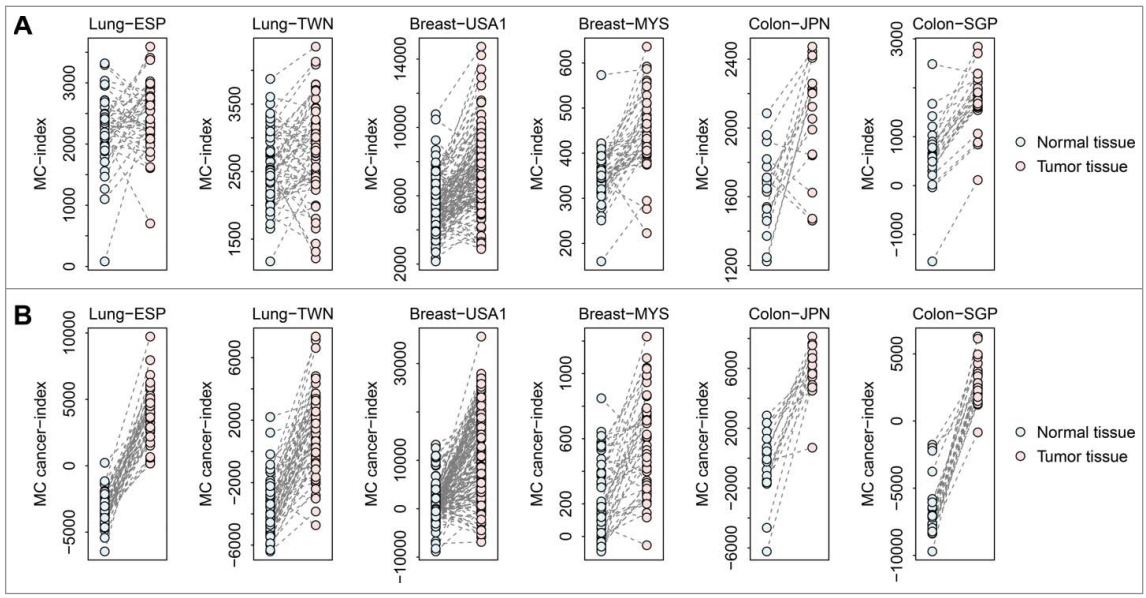

Figure 2. Comparison of MC-index and MC cancer-index between human tumor and normal tissues. Both the MC-index (Panel A) and MC cancer-index (Panel B) were compared between paired tumor and normal tissues from lung, breast, and colon cancer patients, respectively. Six independent human cancer cohorts were analyzed here.

(MCDC) signature (Table 1). To test the predictive power of the MCDC signature, we constructed a scoring system to assign each patient a risk score, representing a linear combination of the MCDC gene expression values weighted by the coefficients obtained from the training data sets (GEO accession: GSE8894, GSE21653, and GSE17536 for lung,[49] breast,[50] and colon[51] cancers, respectively) (see Materials and Methods for details). We speculated that a higher MCDC-based risk score implies a poorer clinical outcome. MCDC-positive ($MCDC^+$) patients were defined as those having risk scores larger than zero whereas the other patients were assigned as MCDC-negative ($MCDC^-$).

We tested the prognostic power of the MCDC-based risk score in independent validation cohorts. For each cancer type, 2 validation data sets were collected: 2 lung cancer cohorts from Japan (Lung-JPN; GEO accession: GSE31210)[52] and Sweden (Lung-SWE; GEO accession: GSE37745),[53] respectively; 2 breast cancer cohorts from Singapore (Breast-SGP; GEO accession: GSE4922)[54] and the United States (Breast-USA2; GEO accession: GSE2034),[55] respectively; and 2 colon cancer cohorts from France (Colon-FRA; GEO accession: GSE39582)[56] and Netherlands (Colon-NLD; GEO accession: GSE33113),[57] respectively. These data sets were chosen based on the availability of recurrence-free survival information. Kaplan-Meier survival curves demonstrated a significantly reduced recurrence-free survival for the $MCDC^+$ patients compared with the $MCDC^-$ ones in all the validation cohorts (log-rank test: $P = 9.8 \times 10^{-4}$ for Lung-JPN; $P = 3.3 \times 10^{-2}$ for Lung-SWE; $P = 1.4 \times 10^{-4}$ for Breast-SGP; $P = 5.5 \times 10^{-3}$ for Breast-USA2;
$P = 4.0 \times 10^{-2}$ for Colon-FRA; $P = 4.4 \times 10^{-3}$ for Colon-NLD) (Fig. 3). Univariate Cox proportional hazards regression also confirmed the relationship between MCDC status and clinical outcome: the $MCDC^+$ patients have a 2.35-, 1.83-, 2.23-, 1.70-, 1.36-, and 4.40-fold increased risk of recurrence in the Lung-JPN, Lung-SWE, Breast-SGP, Breast-USA2, Colon-FRA, and Colon-NLD cohorts, respectively (Table 2). These findings collectively indicate that the MCDC signature is predictive of recurrence-free survival in lung, breast, and colon cancers.

Next, we investigated the performance of the MCDC signature in comparison with standard clinical and pathological factors associated with prognosis in human cancers. For the Lung-JPN cohort, patient age, gender, smoking history, stage, *EGFR*/*KRAS*/*ALK* gene mutation status, and MYC protein levels were considered. For the Lung-SWE cohort, we took age, gender, stage, and WHO performance status into account. For the Breast-SGP cohort, patient age, gender, grade, tumor size, lymph node status, estrogen receptor (ER) status, and *TP53* mutation status were considered. For the Breast-USA2 cohort, ER status were included as covariate. For the Colon-FRA cohort, we considered factors including age, gender, stage, and *BRAF*, *KRAS*, and *TP53* mutation status. For the Colon-NLD cohort, patient age and gender were considered as covariate. Multivariate Cox proportional hazards regression indicates that the MCDC status remained a significant covariate in relation to the clinical and pathological factors in each validation cohorts ($P = 3.8 \times 10^{-2}$ for Lung-JPN; $P = 3.8 \times 10^{-2}$ for Lung-SWE; $P = 2.4 \times 10^{-2}$ for Breast-SGP; $P = 4.0 \times 10^{-3}$ for Breast-

Table 1. The MCDC gene signature.

Gene symbol	Gene title
ACP1	acid phosphatase 1, soluble
AKAP9	A kinase (PRKA) anchor protein (yotiao) 9
ARGLU1	arginine and glutamate rich 1
BCAS2	breast carcinoma amplified sequence 2
BCR	breakpoint cluster region
BTRC	β-transducin repeat containing E3 ubiquitin protein ligase
CCDC59	coiled-coil domain containing 59
CEP57	centrosomal protein 57kDa
CHD4	chromodomain helicase DNA binding protein 4
CNOT4	CCR4-NOT transcription complex, subunit 4
CPSF6	cleavage and polyadenylation specific factor 6, 68kDa
CXCL12	chemokine (C-X-C motif) ligand 12
DDX39A	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39A
DDX6	DEAD (Asp-Glu-Ala-Asp) box helicase 6
DNAJC2	DnaJ (Hsp40) homolog, subfamily C, member 2
EIF3A	eukaryotic translation initiation factor 3, subunit A
EIF5	eukaryotic translation initiation factor 5
ELF2	E74-like factor 2 (ets domain transcription factor)
ENY2	enhancer of yellow 2 homolog (Drosophila)
FEZ2	fasciculation and elongation protein zeta 2 (zygin II)
FYTTD1	42-three domain containing 1
GAS2L3	growth arrest-specific 2 like 3
HDLBP	high density lipoprotein binding protein
HERPUD1	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1
HNRNPU	heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A)
HSPA8	heat shock 70kDa protein 8
IBTK	inhibitor of Bruton agammaglobulinemia tyrosine kinase
IFNGR1	interferon gamma receptor 1
LIMS1	LIM and senescent cell antigen-like domains 1
LRRFIP1	leucine rich repeat (in FLII) interacting protein 1
LTN1	listerin E3 ubiquitin protein ligase 1
LUC7L3	LUC7-like 3 (S. cerevisiae)
MCM4	minichromosome maintenance complex component 4
MRPL13	mitochondrial ribosomal protein L13
NAA15	N(α)-acetyltransferase 15, NatA auxiliary subunit
NEMF	nuclear export mediator factor
NR1H3	nuclear receptor subfamily 1, group H, member 3
NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1
ORC2	origin recognition complex, subunit 2
PDAP1	PDGFA associated protein 1
PDLIM5	PDZ and LIM domain 5
PFDN1	prefoldin subunit 1
PGGT1B	protein geranylgeranyltransferase type I, β subunit
PLLP	plasmolipin
POGZ	pogo transposable element with ZNF domain
PPAT	phosphoribosyl pyrophosphate amidotransferase
PPP1R12B	protein phosphatase 1, regulatory subunit 12B
PPTC7	PTC7 protein phosphatase homolog (S. cerevisiae)
PRKG1	protein kinase, cGMP-dependent, type I
PRPF40A	PRP40 pre-mRNA processing factor 40 homolog A (S. cerevisiae)
PSMC4	proteasome (prosome, macropain) 26S subunit, ATPase, 4
RBM26	RNA binding motif protein 26
RBM4	RNA binding motif protein 4
RBM5	RNA binding motif protein 5
RNF169	ring finger protein 169
RNPC3	RNA-binding region (RNP1, RRM) containing 3
SDAD1	SDA1 domain containing 1
SERF1A	small EDRK-rich factor 1A (telomeric)
SKP2	S-phase kinase-associated protein 2, E3 ubiquitin protein ligase
SMARCA5	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5
SMARCAD1	SWI/SNF-related, matrix-associated actin-dependent regulator of chromatin, subfamily a, containing DEAD/H box 1
SNCG	synuclein, gamma (breast cancer-specific protein 1)

(*Continued on next page*)

Table 1. (*Continued*)

Gene symbol	Gene title
SOCS3	suppressor of cytokine signaling 3
SORBS1	sorbin and SH3 domain containing 1
SOX4	SRY (sex determining region Y)-box 4
SRSF3	serine/arginine-rich splicing factor 3
SSB	Sjogren syndrome antigen B (autoantigen La)
STAU2	staufen, RNA binding protein, homolog 2 (Drosophila)
SVEP1	sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1
SYNCRIP	synaptotagmin binding, cytoplasmic RNA interacting protein
TC2N	tandem C2 domains, nuclear
TGFBR3	transforming growth factor, β receptor III
THOC1	THO complex 1
TMEM38B	transmembrane protein 38B
TOR1AIP1	torsin A interacting protein 1
TRA2B	transformer 2 β homolog (Drosophila)
TRIOBP	TRIO and F-actin binding protein
TRMT6	tRNA methyltransferase 6 homolog (S. cerevisiae)
TTC3	tetratricopeptide repeat domain 3
TTC9C	tetratricopeptide repeat domain 9C
USP7	ubiquitin specific peptidase 7 (herpes virus-associated)
ZC3H15	zinc finger CCCH-type containing 15

USA2; $P = 2.6 \times 10^{-2}$ for Colon-FRA; $P = 1.4 \times 10^{-2}$ for Colon-NLD) (Table 3), which suggests that the MCDC signature is independent of standard clinical and pathological prognostic factors in lung, breast, and colon cancers.

A bioinformatical study by Venet *et al.* points out that most published gene signatures are not significantly better than random gene sets of identical size that are randomly picked up from human genome.[58] To address this issue, we further conducted resampling test for the MCDC signature. We obtained 1,000 random gene signatures by randomly selecting 82 genes from human genome. For each random set of genes, multivariate Cox proportional hazards regression was conducted. The association between each random gene signature and survival was measured by the mean of Cox regression *Z*-score. We found that the mean of *Z*-score of the MCDC signature is significantly larger than that of the random gene signatures (Right-tailed: $P = 0.022$) (Supplementary Fig. S3), which suggests the empirically non-random association between the MCDC signature and survival.

Discussion

For decades, there has been particular interest and speculation as to the physiologic function of mast cells in tumor biology. We know mast cells potentially influence many aspects of tumor biology, including tumor angiogenesis,[5,11] tumor invasiveness,[6] and immunosuppression[1,15]; however, the exact contributions of mast cells in tumorigenesis remain controversial. Particularly, there have been a considerable number of contradictory observations regarding the detrimental or protective roles of mast cells in tumor development.[31] Although elucidating the detailed reasons for these discrepancies is beyond the scope of this study, we have presented a transcriptomic perspective to study the impact of mast cells on shaping tumor microenvironment. Based on the transcriptomic data from WT, $Kit^{W\text{-}sh}$, and $Kit^{W\text{-}sh+}$MC mice, we identified the mast cell–dependent genes, which were deregulated by mast cell deficiency but largely recovered upon mast cell engraftment. To quantify the transcriptomic impact caused by mast cells in tissue microenvironment, a computational algorithm was developed to assign each tissue sample a MC-index based on the rank-weighted expression profile of mast cell–dependent genes, which potentially serves as a proxy of mast cell

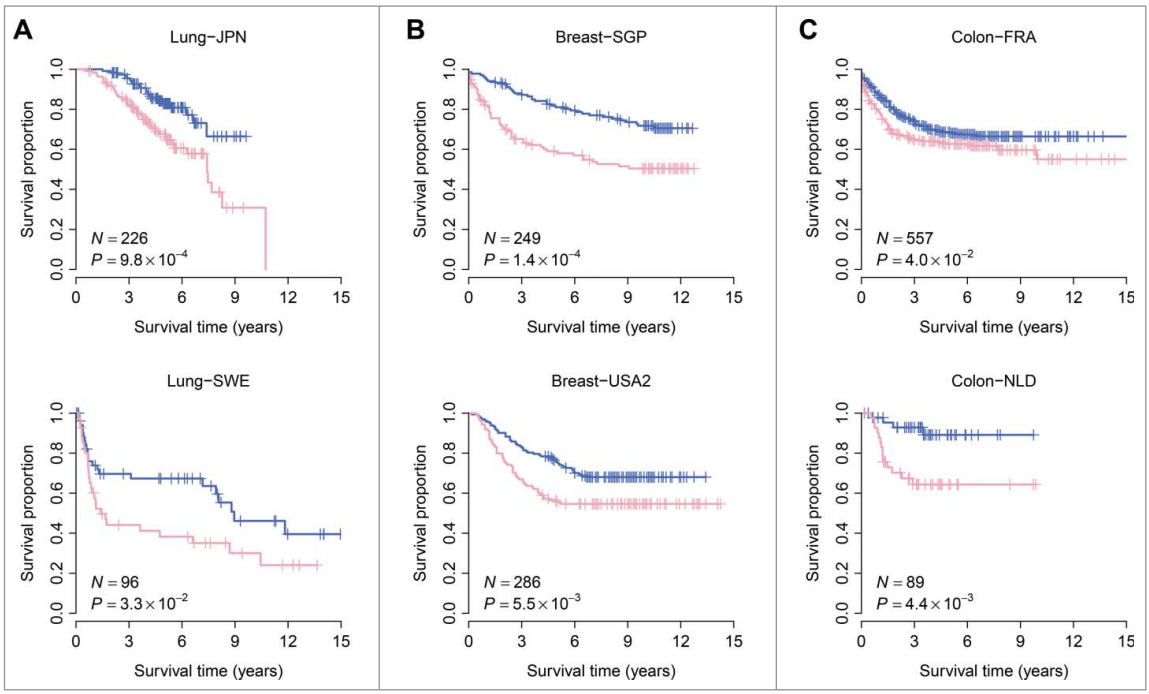

Figure 3. The MCDC signature predicts recurrence-free survival in lung, breast, and colon cancers. Kaplan-Meier curves were presented for lung (Panel A), breast (Panel B), and colon (Panel C) cancer, respectively. Six independent human cancer cohorts were analyzed here. The pink curves are for the $MCDC^{+}$ patients whereas the blue curves are for the $MCDC^{-}$ patients. The *P*-values were calculated by log-rank test.

Table 2. Cox proportional hazards regression of recurrence-free survival by MCDC status.

Cohort	HR	95% CI	*P*-value
Lung-JPN	2.35	(1.39, 3.98)	1.4×10^{-3}
Lung-SWE	1.83	(1.04, 3.23)	3.6×10^{-2}
Breast-SGP	2.23	(1.46, 3.40)	2.0×10^{-4}
Breast-USA2	1.70	(1.17, 2.49)	5.9×10^{-3}
Colon-FRA	1.36	(1.02, 1.83)	4.0×10^{-2}
Colon-NLD	4.40	(1.44, 13.38)	9.1×10^{-3}

Note – HR: hazard ratio; CI: confidence interval

infiltration level in the tissue microenvironment. We indicate that, in both mouse models and human patients, the MC-indices of tumors are statistically higher than those of normal tissues from lung, breast, and colon, respectively. To more precisely assess the contribution of mast cells in shaping the tumor microenvironment, the MC cancer-index was computed for each tissue sample, based on the expression profile of mast cell–dependent cancer (MCDC) genes commonly deregulated in mouse lung, breast, and colon tumors. The difference in MC cancer-index between tumor and normal tissues mirrors the pattern we observed for MC-index in both mouse and human. Based on the MCDC genes, the MCDC signature was developed, which predicts clinical outcomes as an independent covariate in lung, breast, and colon cancers, respectively.

Despite the debate over detrimental/protective roles for mast cells in tumorigenesis, we demonstrate a potential increased

Table 3. Multivariate Cox proportional hazards regression of survival in the validation cohorts.

Cohort	Covariate	HR	95% CI	*P*-value
Lung-JPN	MCDC + vs. −	1.79	(1.01, 3.04)	3.8×10^{-2}
	Age (per year)	1.04	(1.00, 1.08)	4.2×10^{-2}
	Gender male vs. female	0.72	(0.36, 1.42)	3.4×10^{-1}
	Smoking + vs. −	1.50	(0.75, 2.85)	2.7×10^{-1}
	Stage	2.85	(1.68, 4.84)	1.0×10^{-4}
	EGFR/KRAS/ALK mutation + vs. −	0.60	(0.36, 1.01)	5.3×10^{-2}
	MYC level high vs. low	0.94	(0.37, 2.40)	9.0×10^{-1}
Lung-SWE	MCDC + vs. −	1.99	(1.04, 3.81)	3.8×10^{-2}
	Age (per year)	0.99	(0.96, 1.03)	7.3×10^{-1}
	Gender male vs. female	1.01	(0.54, 1.88)	9.8×10^{-1}
	Stage	1.81	(0.86, 3.79)	1.2×10^{-1}
	WHO performance	1.33	(0.97, 1.82)	7.8×10^{-2}
Breast-SGP	MCDC + vs. −	1.84	(1.08, 3.11)	2.4×10^{-2}
	Age (per year)	1.01	(0.99, 1.02)	4.3×10^{-1}
	grade	1.19	(0.78, 1.81)	4.1×10^{-1}
	Tumor size	1.01	(0.99, 1.02)	3.1×10^{-1}
	Lymph node + vs. −	1.50	(0.94, 2.40)	9.2×10^{-2}
	ER + vs -	1.17	(0.62, 2.23)	6.2×10^{-1}
	TP53 mutation + vs. −	1.14	(0.66, 1.96)	6.4×10^{-1}
Breast-USA2	MCDC + vs. −	1.79	(1.20, 2.66)	4.0×10^{-3}
	ER + vs -	1.21	(0.77, 1.91)	4.1×10^{-1}
Colon-FRA	MCDC + vs. −	1.41	(1.04, 1.91)	2.6×10^{-2}
	Age (per year)	1.01	(0.99, 1.02)	3.7×10^{-1}
	Gender male vs. female	1.49	(1.10, 2.03)	1.1×10^{-2}
	Stage	2.71	(2.16, 3.39)	$< 10^{-10}$
	BRAF mutation + vs. −	0.86	(0.47, 1.59)	6.4×10^{-1}
	KRAS mutation + vs -	1.28	(0.93, 1.75)	1.3×10^{-1}
	TP53 mutation + vs. −	1.51	(1.11, 2.04)	7.7×10^{-3}
Colon-NLD	MCDC + vs. −	4.15	(1.34, 12.87)	1.4×10^{-2}
	Age (per year)	0.99	(0.95, 1.02)	4.8×10^{-1}
	Gender male vs. female	0.78	(0.29, 2.05)	6.1×10^{-1}

Note – HR: hazard ratio; CI: confidence interval

trend in MC- and MC cancer-indices in lung, breast, and colon tumors from a transcriptomic perspective, which suggests that, compared with normal tissues, the MC^+ genes are more likely to be upregulated while the MC^- ones tend to be downregulated in tumor microenvironment. In other words, the mast cell infiltration could be increased in lung, breast, and colon tumors compared with that in normal lung, breast, and colon tissues, respectively, suggesting that mast cells might be implicated in tumor development and progression.

The transcriptomic data from $Kit^{W\text{-}sh}$ mice were applied in this study to infer the mast cell–dependent genes. Mice bearing *c-kit* mutations exhibit reduced *c-kit* tyrosine kinase–dependent signaling that results in not only mast cell deficiency but also other phenotypic abnormalities.[27] Therefore, the differential gene expression between $Kit^{W\text{-}sh}$ and WT mice might arise from other abnormalities, not solely attributed to mast cell deficiency. To address this issue, we used $Kit^{W\text{-}sh+}$MC mice to assess to what extent the abnormalities in gene expression of *c-kit* mutant mice can be recovered by mast cell engraftment.[6] Hence, only the genes that were deregulated in mast cell–deficient mice but recovered upon mast cell engraftment were defined as mast cell–dependent genes.

The predictive power of the MCDC signature illustrates the link between the mast cell–dependent genes and prognosis of lung, breast, and colon cancers, which may be a common feature in various cancers. Indeed, *ACP1*,[59] *AKAP9*,[60] *BCAS2*,[61] *CEP57*,[62] *CXCL12*,[63] *EIF5*,[64] *LRRFIP1*,[65] *NUCKS1*,[66] *PFDN1*,[67] *RBM4*,[68] *SKP2*,[69] *SMARCA5*,[70] *SMARCAD1*,[71] *SNCG*,[72] *SOCS3*,[73] *SOX4*,[74] *SVEP1*,[75] *TGFBR3*,[76] *THOC1*,[77] and *USP7*[78] from the MCDC signature are already under investigation regarding cancer pathology or treatment in some capacity. More work is needed to determine whether the other genes in our signature could be exploited for cancer therapy.

Recently, Dwyer et al. proposed a mast cell signature, which consists of 128 genes upregulated in mast cells compared with the other immunocytes.[79] However, we didn't find any overlap between the 128 gene and MCDC signatures, which may be due to the difference in utility between the 2 signatures. The 128 gene signature was designed to differentiate mast cells from the other cells, which was developed upon cell-level gene expression data. In contrast, the MCDC signature was derived from tissue-level transcriptomic analysis and reflects the integrated signal of individual cell types, which implicitly correlates with mast cell infiltration and tumor development. Therefore, it's fairly reasonable that the 128 gene signature fails to differentiate tumors from normal tissues (Supplementary Fig. S4), while the MCDC signature works the other way around.

Although the MCDC signature potentially reflects some common mechanisms shared by different cancers, some mast cell–dependent genes differ substantially in expression pattern among different tumor types (Supplementary Fig. S5), which represent the intrinsic pathological difference among cancers. For example, the top GO terms associated with the mast cell–dependent genes differing between lung and breast tumors, between lung and colon tumors, and between breast and colon tumors are "tube development," "positive regulation of nitrogen compound metabolic

process" and "mammary gland development," respectively (Supplementary Fig. S5).

Understanding the mast cell–dependent transcriptomic pattern may provide therapeutic benefit in cancer treatment. Our study provides a provocative insight into the role of mast cells in cancers. The expression profile of the mast cell–dependent genes potentially serves as a promising proxy of the impact of mast cells on tumor microenvironment although the molecular mechanisms remain unclear. When working cooperatively with known clinical and pathological prognostic factors, the MCDC signature might enhance the prediction accuracy for identifying patients at higher risk for recurrence. However, the real physiologic role of mast cells is more complicated than the transcriptomic data and appears to vary with cancer types. In future study, intensive experimental investigation is apparently needed to validate the exact role of individual mast cell–dependent genes in different cancers.

Materials and methods

Transcriptomic data

Four mouse transcriptomic data sets were included in this study. Firstly, the microarray data of lung RNA from WT, *Kit*$^{W\text{-}sh}$, and *Kit*$^{W\text{-}sh+}$MC mice were obtained from the GEO[28] database (GEO accession: GSE27066; Affymetrix Mouse Genome 430 2.0 Array).[29] We used this data set to filter out the mast cell–dependent mouse genes. Secondly, from the GEO database, we downloaded the gene expression data of both tumor and normal tissues in mouse lung (GEO accession: GSE31013; Affymetrix Mouse Genome 430 2.0 Array),[38] breast (GEO accession: GSE21444; Affymetrix Mouse Genome 430 2.0 Array),[39] and colon (GEO accession: GSE50794; Affymetrix Mouse Genome 430 2.0 Array).[40] These data sets were used to examine the deregulation pattern of the mast cell–dependent genes in mouse tumors.

For human subjects, we applied 18 independent whole-genome gene expression data sets in this study. Firstly, we obtained the microarray data of paired normal and tumor tissues derived from lung, breast, colon, liver, prostate, and thyroid cancer patients from the GEO database. For lung cancer, we included the Lung-ESP (GEO accession: GSE18842; Affymetrix Human Genome U133 Plus 2.0 Array)[41] and Lung-TWN (GEO accession: GSE19804; Affymetrix Human Genome U133 Plus 2.0 Array)[42] cohorts; for breast cancer, we included the Breast-USA1 (GEO accession: GSE70947; Agilent-028004 SurePrint G3 Human GE 8 × 60K Microarray) and Breast-MYS (GEO accession: GSE15852; Affymetrix Human Genome U133A Array)[43] cohorts; for colon cancer, we included the Colon-JPN (GEO accession: GSE22598; Affymetrix Human Genome U133 Plus 2.0 Array)[44] and Colon-SGP (GEO accession: GSE10950; Illumina humanRef-8 v2.0 expression beadchip)[45] cohorts; for liver, prostate, and thyroid cancers, the following 3 data sets were included respectively: GSE14520 (Affymetrix Human Genome U133A 2.0 Array),[46] GSE32448 (Affymetrix Human Genome U133 Plus 2.0 Array),[47] and GSE33630 (Affymetrix Human Genome U133 Plus 2.0 Array).[48] These data sets were used to examine the deregulation profiles of the mast cell–dependent genes in human tumors. To investigate the prognostic power of the mast cell–dependent genes, we constructed training and validation cohorts for lung, breast, and colon cancers, respectively. From the GEO database, we first collected the training data sets with available information on recurrence-free survival for lung (GEO accession: GSE8894; Affymetrix Human Genome U133 Plus 2.0 Array),[49] breast (GEO accession: GSE21653; Affymetrix Human Genome U133 Plus 2.0 Array),[50] and colon (GEO accession: GSE17536; Affymetrix Human Genome U133 Plus 2.0 Array)[51] cancers, respectively. Next, 2 validation cohorts with clinical outcome information were downloaded for each cancer type. For lung cancer, we collected the Lung-JPN (GEO accession: GSE31210; Affymetrix Human Genome U133 Plus 2.0 Array)[52] and Lung-SWE (GEO accession: GSE37745; Affymetrix Human Genome U133 Plus 2.0 Array)[53] cohorts; for breast cancer, we included the Breast-SGP (GEO accession: GSE4922; Affymetrix Human Genome U133A and U133B Arrays)[54] and Breast-USA2 (GEO accession: GSE2034; Affymetrix Human Genome U133A Array)[55] cohorts; for colon cancer, we considered the (Colon-FRA, GEO accession: GSE39582; Affymetrix Human Genome U133 Plus 2.0 Array)[56] and Colon-NLD (GEO accession: GSE33113; Affymetrix Human Genome U133 Plus 2.0 Array)[57] cohorts.

Detecting differential gene expression

Significance analysis of microarrays (SAM),[80] implemented in the *samr* library of the R Statistical Package, was used to identify deregulated genes. False discovery rate was controlled using the q-value method.[81] Transcripts with a fold-change >1.5 and false discovery rate <0.05 were deemed differentially expressed. We limited our analysis to the probes/probesets with unique annotations and removed genes on chromosomes X and Y to avoid the potential confounding sex factor.

Mast cell index and mast cell cancer index

Briefly, mast cell index (MC-index) is the difference in normalized centroid of rank-weighted gene expression between the MC$^+$ and MC$^-$ genes, which is designed to utilize transcriptomic data to assess the impact of mast cells on shaping tissue microenvironment. For a transcriptomic data set with n genes, all genes in each sample are sorted in ascending order according to their expression values. If r_i is the rank of gene i in a sample, the exponential weight (w_i) of gene i can be calculated as:

$$w_i = r_i \cdot e^{r_i / n} \quad (1)$$

For the MC$^+$ genes, let n^+ be the number of the genes and the normalized centroid (C^+) can be calculated as the mean of gene weight across all the MC$^+$ genes (Equation 2). For the complement gene set composed of all the other non-MC$^+$ genes, let $\overline{n^+}$ be the number of the genes and the normalized centroid $(\overline{C^+})$ can be calculated as the mean of gene weight

across all the non-MC$^+$ genes (Equation 3). The index of the MC$^+$ genes (I^+) is simply the difference between the normalized centroid of MC$^+$ and non-MC$^+$ genes (Equation 4).

$$C^+ = \frac{1}{n^+} \sum_{i=1}^{n^+} w_i \quad (2)$$

$$\overline{C^+} = \frac{1}{\overline{n^+}} \sum_{i=1}^{\overline{n^+}} w_i \quad (3)$$

$$I^+ = C^+ - \overline{C^+} \quad (4)$$

Similarly, for the MC$^-$ genes, let n^- be the number of the genes and the normalized centroid (C^-) can be calculated as the mean of gene weight across all the MC$^-$ genes (Equation 5). For the complement gene set composed of all the other non-MC$^-$ genes, let $\overline{n^-}$ be the number of the genes and the normalized centroid ($\overline{C^-}$) can be calculated as the mean of gene weight across all the non-MC$^-$ genes (Equation 6). The index of the MC$^-$ genes (I^-) is the difference between the normalized centroid of MC$^-$ and non-MC$^-$ genes (Equation 7). Finally, the MC-index (I) of each sample is calculated as the difference between I^+ and I^- (Equation 8).

$$C^- = \frac{1}{n^-} \sum_{i=1}^{n^-} w_i \quad (5)$$

$$\overline{C^-} = \frac{1}{\overline{n^-}} \sum_{i=1}^{\overline{n^-}} w_i \quad (6)$$

$$I^- = C^- - \overline{C^-} \quad (7)$$

$$I = I^+ - I^- \quad (8)$$

Mast cell cancer index (MC cancer-index) is designed to assess the impact of mast cells in tumor development. The method to compute MC cancer-index is the same as the procedure to compute MC-index, except for 2 modifications: i) replacing the MC$^+$ genes with the MC$^+$ genes commonly upregulated in mouse lung, breast, and colon tumors; and ii) replacing the MC$^-$ genes with the MC$^-$ genes commonly downregulated in mouse lung, breast, and colon tumors.

Risk score

Based on the gene expression and clinical outcome data from the training data sets (GEO accession: GSE8894, GSE21653, and GSE17536 for lung,[49] breast,[50] and colon[51] cancers, respectively), we conducted univariate Cox proportional hazards regressions to evaluate the association between recurrence-free survival and gene expression for lung, breast, and colon cancers, respectively. A risk score was then calculated for each patient using a linear combination of gene expression weighted by the Wald statistic (ratio of regression coefficient to its standard error)[82-84] as shown below:

$$s = \sum_{i=1}^{n} w_i(e_i - \mu_i) \,/\, \tau_i \quad (9)$$

Here, s is the risk score of the patient; n is the number of genes; w_i denotes the Wald statistic of gene i; e_i denotes the expression level of gene i; and μ_i and τ_i are the mean and standard deviation of the gene expression values for gene i across all samples, respectively. Patients were then divided into high-risk and low-risk groups with zero as the cutoff. We speculated that a higher risk score implies a poorer clinical outcome.

Disclosure of interest

The authors report no conflict of interest.

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