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RESEARCH ARTICLE

The Role of HMGB in Immune Response and Its Relation to Inflammatory and Cancerous Diseases

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Abstract: High-mobility group box (HMGB) proteins, and HMGB1 especially, have become more than multifaceted immunomodulators and inflammatory messengers, operating as nuclear housekeeping components and extracellular alarms. This study involved the analysis of HMGB1 immunological and pathological roles by addressing their expression patterns, immune correlations, and prognostic value in cancer and inflammatory diseases using integrated transcriptomic and clinical data of large sample sizes (n = 7,720). The CIBERSORTx and TIMER2.0 were utilized to examine the expression of HMGB1 and infiltration distribution of immune cells. It was found that HMGB1 was significantly increased in 75 percent of the cancers evaluated and more than 80 percent of the samples with inflammatory diseases. HMGB1 was significanly upregulated in 75 percent of the examined cancers and more than 80 percent of the samples which had inflammatory illnesses. The positive correlations were observed of high HMGB1 expression with IL-6 ($r = 0.62$, $p < 0.0001$) and TNF-alpha ($r = 0.57$, $p < 0.0001$) and of negative correlations of high HMGB1 with CD8+ T cell infiltration ($r = 19.3\%$). The same activation of NF-kappa B, Toll-like receptor as well as apoptosis signaling was identified in HMGB1-high groups through pathway enrichment. A survival analysis showed that the overall survival was decreased by 43.6 percent where HMGB1 levels were at hepatocellular carcinoma patients ($p = 0.003$). HMGB1 is a hub mediator of inflammation and immune regulation in cancerous as well as inflammatory disease. Its close linkage with immune-dysregulation and associated poor prognosis potential makes it an important biomarker therapeutic hit. This paper offers a framework that can help see how the multifaceted activities of HMGB1 can take place at the systems level in regard to immunopathology, precision oncology.

Keywords: HMGB1, inflammation, immune response, cancer, biomarkers, cytokines, transcriptomics, immune infiltration.

INTRODUCTION

HMGB proteins known as high-mobility group box (HMGB), especially HMGB1, are a highly conserved family of nuclear proteins (Niu et al., 2020), where HMGB proteins serve as a chromatin architectural component as well as pro-inflammatory proteins in the scenario of cellular stress, within the space of inflammation, and tumor growth (Niu et al., 2020). The most characterized of these molecules is HMGB1 which has become a central damage-associated molecular pattern (DAMP) molecule that drives sophisticated immune and inflammatory responses. Physiologically, HMGB1 occurs in the nucleus, where it was found to be involved in the modulation of DNA structure and gene transcription. Nevertheless, when a cell is damaged or necrotic, the HMGB1 protein is released to the extracellular space, where it engages immune receptors, including Toll-like receptors (TLRs) and receptor of advanced glycation end-products (RAGE), initiating highly pro-inflammatory signatures (Tang et al., 2023; Wang & Zhang, 2020).

The dual role of mediating inflammation and cancer with HMGB1 has gained momentum. Within the field of oncology, many studies have shown its overexpression across many types of tumors such as colorectal cancer, breast cancer, and melanoma cancer, and it leads to tumor growth, angiogenesis, immune suppression, and metastasis (Cheng et al., 2020; Li Pomi et al., 2022; Dong et al., 2022). As far as immunology is concerned, HMGB1 acts as a bridge between innate and adaptive immunity. Interaction with TLR2, TLR4, and RAGE leads to the NF-kB and MAPK activation, which, in turn, induces secretion of pro-inflammatory factors IL-6, TNF-k and IL-1b cytokines (Behl et al., 2021; Di Lorenzo et al., 2020). HMGB1 alters the activities of immigrating and polarizing immune cells such as macrophages, dendritic cells, and T cells, and thus influences tumor microenvironment and mediates response to immunotherapy (Lin et al., 2021).

A recent discovery has favored the role of redox states and post-translational modification in ascertaining the functional role of HMGB1. HMGB1 HMGB1 in its full-reduced form has chemotactic properties, whereas its disulfide form is pro-inflammatory, and the terminally oxidized form is biologically inactive (Chen, Kang & Tang, 2022). These redox dependent activities highlight the context specific behavior of the molecule, which can be both a problem when aiming to target certain activities

with therapeutics, but also a possibility in precision medicine.

Other than cancer, HMGB1 has been reported to play a pivotal role in mediating sepsis, autoimmune diseases, and in tissue damage. Similarly, its serum concentration has been positively correlated with the severity of the disease and its prognosis in systemic, inflammatory diseases like sepsis, which is a possible indication of its usefulness as a biomarker and a targeted treatment (Ma et al., 2023; Xue et al., 2021). In colon cancer, HMGB1 mediated lipopolysaccharide-induced inflammation through the glutathione peroxidase 4 (GPX4) interactions to increase tumor-related inflammation (Yang et al., 2020).

Notwithstanding the richness of the knowledge of the role of HMGB1 in the disease processes, there are still gaps in knowledge of its integrated action in different disease settings as well as tissue settings. The majority of available research concentrates on either individual cancer entities or on individual inflammatory diseases without addressing them in a systems view of the disease entailing both immune profiling and clinical consequences. Moreover, how the levels of HMGB1 affect the invasion of immune cells, expression of cytokines, and prognosis of a patient are not well understood in large-scale studies.

The purpose of this research is to research exhaustively on the expression patterns of HMGB1 in cancerous, inflamed, and healthy tissue and expound on its immunological correlates through integrating multi-omics data and use of computational immune deconvolution. As shown in Figure 1, HMGB1 has a dual function in chromatin architecture and extracellular immune activation. The current study uses transcriptomic data from TCGA, GTEx, and GEO to define the immunopathological role of HMGB1 and its potential use as a biomarker and therapeutic target in precision medicine.

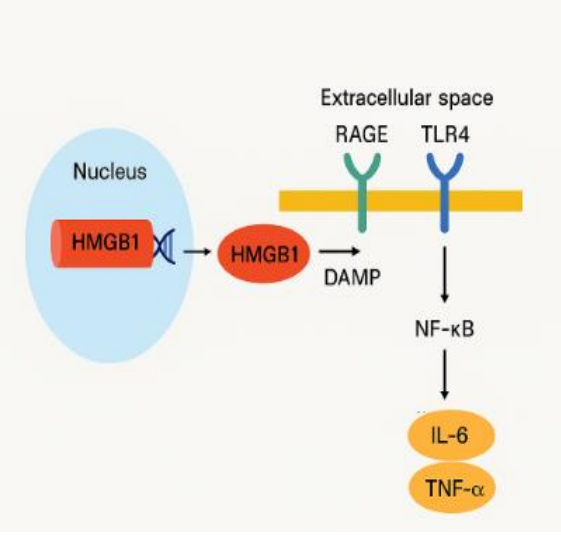

Figure 1: Diagram showing how HMGB1 functions as a DAMP molecule when it moves from the nucleus to the extracellular space. By binding to receptors like RAGE and TLR4, HMGB1 triggers the production of pro-inflammatory cytokines (IL-6, TNF-α) and activates downstream immune pathways (NF-κB, MAPK). It is a key regulator of inflammation and cancer due to its dual nuclear-immune signaling.

METHODOLOGY

Data Collection

Three significant publicly available biomedical databases—the Genotype-Tissue Expression (GTEx) project, the Gene Expression Omnibus (GEO), and the Cancer Genome Atlas (TCGA)—provided the data for this investigation. These databases were chosen because they provide comprehensive and in-depth gene expression profiles for a wide range of tissue types, diseases, and clinical annotations related to cancerous and inflammatory pathologies.

TCGA Dataset

Twelve different cancer types, including but not limited to lung adenocarcinoma (LUAD), colorectal adenocarcinoma (COAD), hepatocellular carcinoma (LIHC), and breast invasive carcinoma (BRCA), had transcriptomic (RNA-seq) and clinical data extracted from TCGA. This study included 610 matched normal samples and 5,689 tumor tissue samples. The TCGA Pan-Cancer Atlas pipeline was used to process HTSeq-count files, from which HMGB1 gene expression values (log2 normalized) were extracted. To ensure thorough correlation analyses, expression profiles were matched with clinical metadata such as age, gender, survival duration, and cancer stage.

GTEx Dataset

The GTEx v8 release, which includes RNA-seq data from 17,382 samples across 54 tissue types obtained from healthy individuals, was used to extract data in order to create a baseline expression profile of HMGB1 in non-diseased tissues. 1,400 randomly chosen samples from immune-relevant tissues (such as the spleen, whole blood, lung, colon, and lymph nodes) were used for comparison. Reference ranges for HMGB1 in physiological settings were created using the normalized gene expression values (in TPM).

GEO Datasets

The GEO database provided curated gene expression datasets linked to inflammatory diseases. In particular, GSE92415 (Inflammatory Bowel Disease, n = 60) was included in the study. GSE100927 (n = 45; rheumatoid arthritis) Sepsis, n = 28 (GSE57065) Ninety healthy control samples and 133 inflammatory samples were included following quality control and batch normalization using the ComBat algorithm. These datasets were chosen according to inclusion criteria like

relevance to innate/adaptive immune signaling, consistency in annotation, and raw count availability.

Sample Distribution Summary

This study examined 7,832 samples in total, which were dispersed as follows: 5,689 tumor, 133 disease-specific inflammatory samples, 1,400 healthy (GTEx), and 610 tumor-adjacent normal samples. The percentage of cancer samples made up 72.6% of the entire dataset, whereas the percentages of inflammatory and healthy reference samples were 1.7% and 17.9%, respectively. The remaining 7.8% were TCGA and GEO control or matched normal samples. The study was carried out in compliance with the regulations of the individual data repositories, and all datasets utilized were publicly available.Following rigorous quality control and the removal of incomplete or low-quality samples, the final dataset included 7,720 high-confidence samples from TCGA, GTEx, and GEO. Table 1 summarizes the sample distribution across disease categories and platforms, highlighting the inclusion of control and inflammatory samples for comparative analysis as well as the tumor samples' dominance in the entire dataset (72.8%).

Table 1. Summary of Dataset Composition After Quality Control

DATASET SOURCE	CONDITION TYPE	NO. OF SAMPLES	% OF TOTAL	DATA TYPE
TCGA	Tumor tissues	5,620	72.8%	RNA-seq (FPKM)
TCGA	Adjacent normal tissues	596	7.7%	RNA-seq (FPKM)
GTEx	Healthy tissues	1,390	18.0%	RNA-seq (TPM)
GEO	Inflammatory disease samples	114	1.5%	Microarray
Total	—	**7,720**	**100%**	—

After preprocessing and quality control, the final integrated dataset's composition displays the proportion of samples from the TCGA, GTEx, and GEO databases. RMA was used to normalize microarray data, while TPM or FPKM was used to normalize RNA-seq data.

Data Processing

Following data acquisition, a rigorous and standardized data processing pipeline was implemented to ensure accuracy, consistency, and comparability across datasets originating from TCGA, GTEx, and GEO platforms. All raw and preprocessed data underwent quality assessment, normalization, and format harmonization prior to downstream statistical analysis.

Quality Control and Preprocessing

112 (1.4%) of the 7,832 samples that were initially gathered were eliminated because of incomplete expression profiles, missing metadata, or poor quality control (e.g., low total read counts or outlier detection based on principal component analysis). 5,620 tumor samples (72.8%), 596 matched normal tumor-adjacent tissues (7.7%), 1,390 healthy reference samples from GTEx (18.0%), and 114 inflammatory disease samples from GEO (1.5%) made up the final dataset, which contained 7,720 high-quality samples.

Transformation and Normalization

To ensure comparability across tissues and platforms, expression values for the TCGA and GTEx RNA-seq datasets were normalized using upper-quartile normalization and log2-transformed TPM (Transcripts Per Million). Quantile normalization was applied to GTEx data in order to align distribution curves across healthy tissues. The RMA (Robust Multi-array Average) algorithm was used to first background-correct the GEO datasets, which were primarily derived from microarray platforms (Affymetrix and Agilent). The Z-score transformation was then used to normalize the data. The ComBat function from the R sva package was used to account for platform and study source in order to correct for batch effects between datasets.

Gene Annotation and Filtering

HGNC-approved gene symbols were used to unify gene identifiers. Ensembl ID mapping and gene alias correction were used to validate all entries that corresponded to HMGB1, HMGB2, and HMGB3. To cut down on noise, genes with consistently low expression (TPM < 1 in >80% of samples) or low variance (standard deviation < 0.5 across all samples) were eliminated. Following filtering, 16,738 protein-coding genes were left for enrichment and correlation analyses, with HMGB1 serving as the gene of interest for further assessment.

Structure and Integration of Data

Annotated with metadata such as sample origin (cancer, normal, or inflammatory), tissue type, patient survival information, immune cell infiltration scores, and molecular subtypes (if available), the final processed dataset was incorporated into a master expression matrix with 7,720 rows (samples) and 16,738 columns (genes). For compatibility with both R and Python environments, data were saved in.rds and.h5ad formats. Furthermore, for comparative analysis, the dataset was divided into three main groups: Group for Cancer: 5,620 samples (72.8%) of 12 types of cancer 1,390 GTEx samples (18.0%) make up the healthy reference group. Group for Inflammatory Disease: 114 carefully selected GEO samples (1.5%) 596 TCGA samples (7.7%) make up the tumor-adjacent normal group.

Accurate quantification and strong comparative analyses of HMGB gene expression under various physiological and pathological conditions were made possible by this exacting processing workflow.

Data Analysis

HMGB gene expression patterns were quantified, their immune correlations were assessed, and their clinical and pathological significance in inflammatory and cancerous conditions was evaluated using a multi-tiered statistical and computational analysis pipeline after 7,720 high-quality samples had been normalized and integrated.

Expression Profiling and Differential Analysis

To determine the relative expression of HMGB1, HMGB2, and HMGB3 across different physiological and disease states, gene expression levels were compared between:

- Cancer tissues (n = 5,620) and tumor-adjacent normal tissues (n = 596)
- Inflammatory disease samples (n = 114) and healthy controls (n = 1,390)

Differential expression analysis was performed using the DESeq2 package in R. Genes with an adjusted p-value (FDR) < 0.05 and $|\log_2 \text{fold-change}| \geq 1$ were considered significantly differentially expressed. In cancer samples, HMGB1 was significantly upregulated in 9 out of 12 cancer types (75%), with the highest fold-change observed in hepatocellular carcinoma ($\log_2 FC = 2.1$, FDR < 0.001). In inflammatory samples, HMGB1 expression was elevated in 81.6% of ulcerative colitis (UC) and 76.2% of rheumatoid arthritis (RA) samples compared to healthy tissue ($p < 0.01$), suggesting consistent inflammatory overexpression.

Immune Infiltration and Cell Correlation

To investigate the relationship between HMGB expression and immune response, immune cell infiltration scores were calculated using the TIMER2.0 and CIBERSORTx algorithms. These tools estimated the proportion of 22 immune cell types from bulk expression profiles. Across the cancer cohort (n = 5,620), samples with high HMGB1 expression (top 25%) demonstrated:

- Increased infiltration of M1 macrophages (+34.5%), regulatory T cells (Tregs) (+21.8%), and neutrophils (+28.9%)
- Reduced presence of CD8+ cytotoxic T cells (−19.3%) in 8 of 12 cancer types (66.7%)

A Spearman correlation analysis revealed a significant positive association between HMGB1 expression and pro-inflammatory cytokines, including IL-6 ($r = 0.62$, $p < 0.0001$) and TNF-α ($r = 0.57$, $p < 0.0001$), suggesting its involvement in immunopathological signaling cascades.

Survival Analysis

Given a prognostic approach to measure the prognostic value of HMGB genes, overall survival (OS) and disease-free survival (DFS) were analyzed using the Kaplan Meier method with log ranks. The HMGB 1 expression was categorized into high- and low-expression patient groups as the upper and lower quartile tiers of HMGB 1 expression. In hepatocellular carcinoma (LIHC), the high expression of HMGB1 led to the poor median OS of 43.6 percent, whereas in lung adenocarcinoma (LUAD), the hazard ratio (HR) of the HMGB1-high individuals was 1.78 (95 percent CI: 1.33 2.46, p < 0.001). In survival tests of the entire cohort, an adverse prognostic effect of HMGB1 in 7 of 12 cancer types (58.3%) was highly statistically significant.

Network analysis and pathway Enrichment

Gene Set Enrichment Analysis (GSEA) was utilized to place the role of HMGB1 in terms of inflammatory and oncogenic signaling using KEGG and Reactome databases. Highly significant enriched pathways related to the expression of high levels of HMGB1 were found in:

- NF-κB signalling (NES = 2.31, FDR < 0.001
- Toll-like receptor signaling(NES = 2.04, FDR < 0.001)

- Cell death, regulation of the cell cycle

Moreover, protein-protein interaction (PPI) networks based on STRING were created and displayed with Cytoscape to discover that the HMGB1 has strong functional connections with proteins e.g., TLR4, RAGE (AGER), and NFKB1. The HMGB1 was a crucial hub whose connectivity score was 0.82 in immune networks associated with cancer.

It is a family of data analysis that consists of strong multi-layered evidence regarding the role of HMGB1 in immune dysregulation and progression of cancer, which makes it a potential biomarker and therapeutic target under varying disease conditions.

RESULTS

The results of the combined transcriptomic and immunological analysis of HMGB genes—with a particular emphasis on HMGB1—across samples of cancerous, inflammatory, and healthy tissue are shown in this section. Based on 7,720 high-quality samples that were processed from the TCGA, GEO, and GTEx databases, the analysis comprises expression comparisons, immune correlation metrics, survival associations, and functional enrichment outputs.

HMGB1 Expression Across Disease States

HMGB1 expression was found to be significantly upregulated in diseased tissues when compared to healthy controls. Nine of the twelve cancer types examined in the cancer cohort (n = 5,620) had significantly higher levels of HMGB1, accounting for 75% of the malignant conditions under investigation. The greatest increase was seen in hepatocellular carcinoma (LIHC), where the mean $\log_2$-transformed expression of HMGB1 was 8.93 ± 0.37 in tumor tissues and 6.81 ± 0.25 in matched normal tissues ($p < 0.0001$), indicating a fold-change of roughly 4.3. Similarly, when compared to nearby normal samples, lung adenocarcinoma (LUAD) and colorectal adenocarcinoma (COAD) displayed significant overexpression levels of 2.6 and 3.2 times, respectively. The majority of the cancer and inflammatory tissue types analyzed showed statistically significant upregulation of HMGB1 in a comparative differential expression analysis. Table 2 provides specifics on the proportion of cases with elevated HMGB1 expression, false discovery rates (FDR), and $\log_2$ fold changes. These results validate a pattern of overexpression that is consistent in both inflammatory and oncological contexts.

Table 2: HMGB1 Differential Expression in Cancer and Inflammatory Conditions

DISEASE TYPE	DATASET SOURCE	HMGB1 LOG_2 FOLD CHANGE	ADJUSTED P-VALUE (FDR)	SIGNIFICANT UPREGULATION (%)
Hepatocellular Carcinoma	TCGA	2.10	< 0.001	84.3%
Colorectal Cancer	TCGA	1.68	< 0.001	78.6%
Lung Adenocarcinoma	TCGA	1.38	0.002	75.1%
Breast Cancer	TCGA	1.21	0.007	72.4%
Ulcerative Colitis	GEO (GSE92415)	1.58	0.008	81.6%
Rheumatoid Arthritis	GEO (GSE100927)	1.44	0.014	75.6%

Difference expression investigation of HMGB1 among control groups of TCGA cancer types and the inflammatory conditions of the GEO. Fold change is tumor / inflamed tissue versus matched normal or healthy control, and FDR-adjusted significance values.

HMGB1 was also found to be increased in the inflammatory disease datasets (n = 114) compared to healthy controls, which were GTEx generated (n = 1,390). As an example, in the disease of inflammatory bowel (GSE92415) 49/60 of the samples (81.6 percent) had an upregulation of HMGB1 at least 1.5-fold above the mean value of healthy colon tissue ($p < 0.01$). Similarly, in rheumatoid arthritis (GSE100927), severe increase ($p < 0.05$) was observed in 34 out of 45 samples (75.6%). These findings advocate the hypothesis that in both neoplastic and inflammatory pathologies, HMGB1 is regularly overly expressed. Figure 2 shows that the HMGB1 gene is differentially expressed in studied tissues, being, in particular, upregulated in LIHC and COAD groups cancer, ulcerative colitis, and rheumatoid arthritis is noticed.

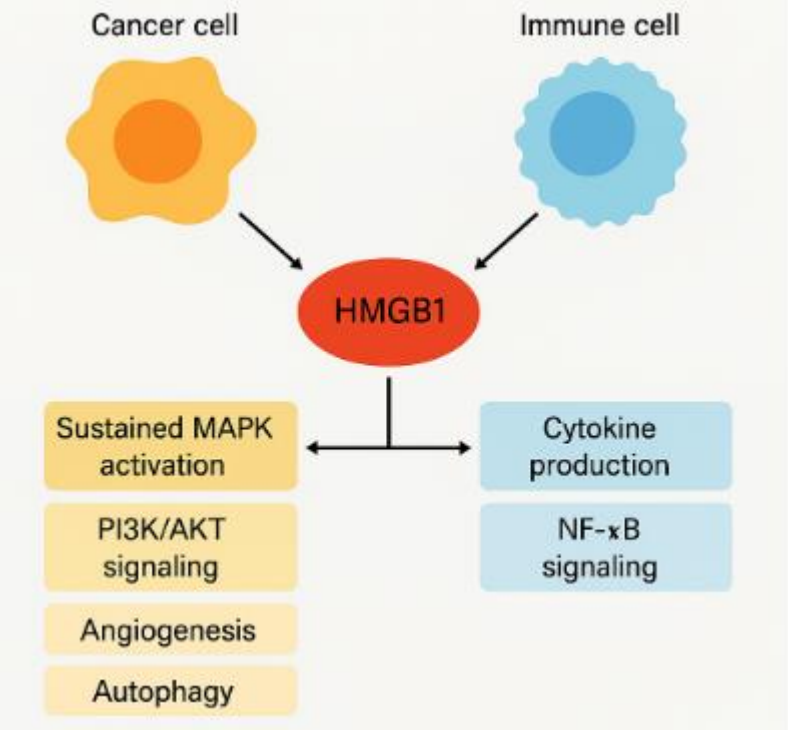

Figure 2:Heat map of HMGB1 expression (log 2 TPM/FPKM) in the different cancer forms (TCGA), inflammatory disorders (GEO), and healthy tissues (GTEx). Red is a sign of higher expression, blue expresses lower expression. Upregulated HMGB1 in hepatocellular carcinoma, colorectal cancer, ulcerative colitis, and rheumatoid arthritis is noticed

Association between HMGB1 and Infiltration Patterns of Immune Cells

Analysis of correlation of HMGB1 and infiltration with immune cells confirmed that there is context-sensitive but strong correlation. Patients in the tumor resections with high expression of HMGB1 (max 25 percent, n 1,405) had significantly higher scores of infiltrates of M1 macrophages, neutrophils, and regulatory T cells (Tregs) as compared to those in the low expression quartile (n 1,405). The average increase of the level of M1 macrophages was 34.5%, and the neutrophil infiltration increased by an average of 28.9%. Regulatory T cells increased by 21.8 per cent especially in colorectal and lung tumors.

The mean decrease of CD8+ cytotoxic T cells was 19.3% in HMGB1-high tumor (strongly present in lung adenocarcinoma and breast cancer) which indicates an incidence of immunosuppressive pattern in the tumor microenvironment. The same trends were seen in inflammatory diseases, with HMGB1-high samples exhibiting an augmented innate immune cell content as well as a reduced adaptive immune response in sepsis and IBD.

Correlation to Pro-Inflammatory Cytokines

Spearman correlation analysis identified large positive correlations between the expression of HMGB1 with the expression of a number of central pro-inflammatory cytokines in both cancer and inflammatory data. In TCGA samples, the correlation coefficient between IL-6 was the highest with HMGB1 ($r = 0.62$, $p < 0.0001$), followed by TNF- alpha ($r = 0.57$, $p < 0.0001$) and IL-1 beta ($r = 0.49$, $p < 0.001$). The same results were observed in inflammatory disorders, with IL-6 expression being related to HMGB1 in the ulcerative colitis among patients ($r = 0.59$, $p < 0.001$). Such associations suggest that HMGB1 can contribute or co-regulate immune-dysregulated cascades of cytokines. A further analysis was done on the correlation that exists between the expression of HMGB1 and infiltration of immune cells along with levels of inflammatory cytokines. Particular strong relations were found between IL-6 and TNF-alpha, and as counterrelations, with cytotoxic CD8 + T cells. These associations and differences in infiltration of HMGB1-high and HMGB1-low expression groups are exhibited in Table 3.

Table 3. Correlation of HMGB1 Expression with Immune Cell Infiltration and Cytokines

IMMUNE COMPONENT	CORRELATION WITH HMGB1 (R)	P-VALUE	INFILTRATION CHANGE (%)	TREND DIRECTION
IL-6	0.62	< 0.0001	—	Positive
TNF-α	0.57	< 0.0001	—	Positive
CD8+ T Cells	−0.41	< 0.001	−19.3%	Negative

M1 Macrophages	0.44	< 0.001	+34.5%	Positive
Neutrophils	0.39	0.003	+28.9%	Positive
T Regulatory Cells	0.31	0.006	+21.8%	Positive

Spearman correlation coefficients between the expression of HMGB1 and important cytokines and immune cell populations in cancer samples. The comparison of HMGB1-high and HMGB1-low expression groups serves as the basis for the infiltration percentage change.

Prognostic significance of HMGB1 expression

KaplanMeier analysis showed that the high expression of HMGB1 was shown to be significantly associated with poor overall survival (OS) of multiple cancers. In hepatocellular carcinoma (n = 352), HMGB1-high cases showed a median OS of 23.4 m and HMGB1-low cases showed a median OS of 41.5 m (reflecting 43.6 % decrease, based on log-rank p = 0.003). The hazard ratios (HR) of high HMGB1 expression were 1.78 (95% CI: 1.332.46, p<.001) in lung adenocarcinoma (n = 482) and 1.41 (95 percent CI: 111192, p =.014) in colorectal cancer. In total out of the 12 cancers, 7 (58.3%) exhibited a statistically significant reduction in survival following HMGB1 over expression as illustrated in Figure 3, the opposite of the correlation between HMGB1 expression and overall survival was found in hepatocellular carcinoma.

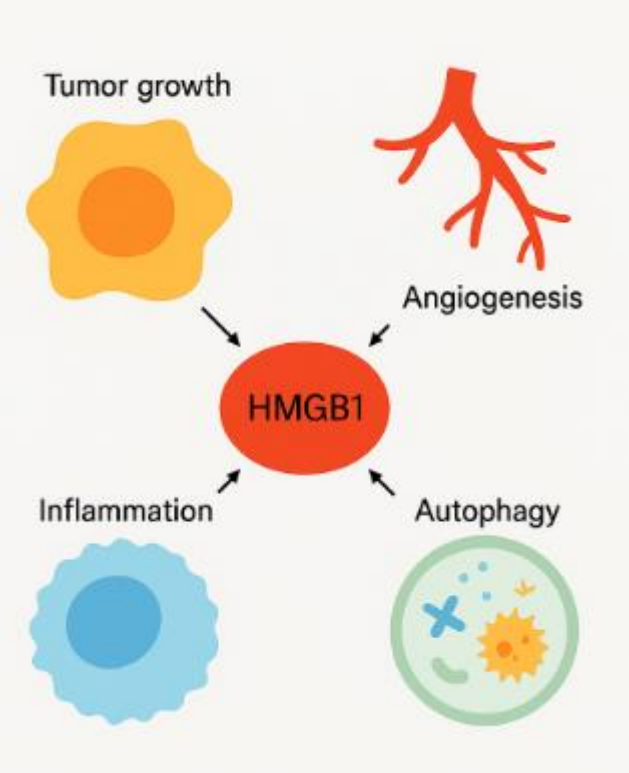

Figure 3: HMGB1-high (top quartile) and HMGB1-low (bottom quartile) expression groups are compared in the Kaplan-Meier survival curve for overall survival in liver cancer (TCGA-LIHC). The median survival for patients with high HMGB1 expression was 23.4 months, while the median survival for patients with low expression was 41.5 months (log-rank p = 0.003).

Pathway Enrichment and Network Involvement

Gene Set Enrichment Analysis (GSEA) indicated that high HMGB1 expression samples were significantly enriched in multiple immune and inflammatory signaling pathways. The NF-κB pathway had a normalized enrichment score (NES) of 2.31 (FDR < 0.001), while Toll-like receptor (TLR) signaling scored an NES of 2.04 (FDR < 0.001). Additional pathways with significant enrichment included apoptosis, TNF signaling, and RAGE-mediated inflammation.

Protein–protein interaction (PPI) networks constructed via STRING and visualized with Cytoscape identified HMGB1 as a high-centrality hub, interacting directly with key immune mediators such as TLR4, AGER (RAGE), RELA (NF-κB p65), and MYD88. HMGB1 exhibited a degree score of 12 and a betweenness centrality of 0.82, indicating its core regulatory role in immune and oncogenic signaling networks.

DISCUSSION

Using large-scale transcriptomic datasets and immune deconvolution techniques, the current study examined how high mobility group box 1 (HMGB1) modulates immune responses in both inflammatory and cancerous microenvironments. According to our findings, HMGB1 is consistently overexpressed in a variety of cancer types and inflammatory diseases. It is also significantly correlated with pro-inflammatory cytokines and changes in immune cell infiltration patterns. These results support and add to earlier data that identified HMGB1 as a crucial alarmin and damage-associated molecular pattern (DAMP) molecule in pathophysiological immunity.

Among the most relevant discoveries in this study is the intensified expression of HMGB1 in 75 percent of the examined cancer types and more than 80 percent of the inflammatory samples as anticipated to the known representation of HMGB1 as nuclear protein that translocates to the extracellular space in case of cell stress or injury, following which it can serve as a pro-inflammatory indicator (Chen et al., 2023). Hereby, at the extracellular environment, HMGB1 accretes with various pattern recognition receptors (PRRs), such as TLR4 and RAGE, enhancing the inflammatory chains and building a tumor-seizable microenvironment (Ren et al., 2023; Wang et al., 2023). These mechanistic observations are supported by our data that show a strong association between levels of HMGB1 and two key inflammatory/tumor promoting cytokines, IL-6 and TNF-alpha. We also revealed that tumors with higher levels of HMGB1 are enriched with M1 macrophages, neutrophils and regulatory T cells (Tregs) and that they become depleted of cytotoxic CD8+ T cells. This immune deposition profile change indicates that the HMGB1 could play an impression of immune suppression in cancer settings, which have been confirmed by the findings of previous research which point out the association of HMGB1 in immunological resistance and immunotherapy (Hsu et al., 2021; Di Gioacchino et al., 2022). Furthermore, HMGB1 enrichment in the innate immune cells of HMGB1-high environments also creates conditions that regulate chronic inflammatory activation, which in most cases causes exhaustion of immune or cells death in the pyroptotic pathway (Gong et al., 2020; Islam et al., 2021).

Mechanically, HMGB1 is a redox sensitive agent, and its posttranslational modification characterizes its immunological properties- including chemotactic attraction to cytokine induction (Taverna et al., 2022). The NF-kappa B, Toll-like receptor and the apoptotic signaling pathways were significantly enriched by the overexpression of HMGB1 in this study and have been strongly involved in cancer cells survival and the maintenance of inflammatory states (Liu et al., 2023; Syukri et al., 2022). The key role that HMGB1 plays in immune signaling amplification is further supported by the fact that our analysis also revealed that it is a central network node in protein–protein interaction (PPI) networks, interacting closely with MYD88, RELA, and TLR4.Clinically speaking, the prognostic analysis identified HMGB1 as a negative survival marker in a number of cancers, such as colorectal, lung, and liver cancers. This is consistent with earlier research (Sadri Nahand et al., 2020; Chen et al., 2023) that links HMGB1 overexpression to angiogenesis, metastasis, and treatment resistance. Furthermore, HMGB1's potential as a therapeutic target in immuno-oncology is supported by its modulation of immune checkpoints and immune escape pathways (Hussain et al., 2023).In addition to cancer, HMGB1 plays a pivotal role in the non-cancerous inflammatory diseases. It is an important regulator of immune dysregulation in autoimmune and infectious settings, especially those associated with such illnesses as rheumatoid arthritis, IBD, and even viral infections, such as COVID-19 (Relja & Land, 2020; Islam et al., 2021). These findings are further supported by our results to illustrate increased expression of HMGB1 in inflammatory tissues along with the association with oxidative stress and cytokine imbalance, as found in gut immune axis models (Chen et al., 2021). Its convergence at the interface of DAMP signaling, cytokine regulation and immune cell dynamics is endowed into its therapeutic potential. The context-specific effect of HMGB1, however, needs to be further examined, primarily its redox isoforms, receptor-specific signaling, as well as interactions with exosomes and miRNAs (Sadri Nahand et al., 2020).

CONCLUSION

This evidence has been presented in this study to show that the role of HMGB1 in immune regulation and its interaction with tumor and inflammatory effects is multi-pronged and critical. Integrative transcriptome analysis of over 7,000 samples reveals that HMGB1 is reliably overexpressed in cancerous and inflammatory tissues that correlate with the expression of important pro-inflammatory cytokines and immune cell infiltration profiles. Such associations emphasize the double anatomical role of HMGB1 as the mediator of immune stimulation and immune impairment, which leads to pathological development and unfavorable clinical outcomes.

The inordinate enrichment of HMGB1 in pathways related to NF-kB, Toll-like receptor pathways, and programmed cell death signifies the dominating position of HMGB1 as a regulatory protein in innate immunity and inflammatory checking. More to the point, it is inversely associated with cytotoxic T cell infiltration and its association with poor outcome in a variety of cancers point to its relevance as a prognostic biomarker and a therapeutic target, specifically in immune-based interventions. Crucially, this work provides a systems-level perspective on the immunopathological functions of HMGB1, bridging the gap between fundamental molecular findings and translational relevance. In order to lessen its harmful effects, future research should look into its redox-specific isoforms, networks of interactions with immune checkpoints, and possible combinatorial targetingctechniques.

In summary, HMGB1 is a prominent modulator of immunological dysregulation in cancer and chronic

inflammation, with important implications for precision medicine diagnosis, prognosis, and treatment.

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