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Review

Recent Computational Approaches in Understanding the Links between Molecular Stress and Cancer Metastasis

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Abstract: In the modern era of medicine, advancements in data science and biomedical technologies have revolutionized our understanding of diseases. Cancer, being a complex disease, has particularly benefited from the wealth of molecular data available, which can now be analyzed using cutting-edge artificial intelligence (AI) and information science methods. In this context, recent studies have increasingly recognized chronic stress as a significant factor in cancer progression. Utilizing computational methods to address this matter has demonstrated encouraging advancements, providing a hopeful outlook in our efforts to combat cancer. This review focuses on recent computational approaches in understanding the molecular links between stress and cancer metastasis. Specifically, we explore the utilization of single-cell data, an innovative technique in DNA sequencing that allows for detailed analysis. Additionally, we explore the application of AI and data mining techniques to these complex and large-scale datasets. Our findings underscore the potential of these computational pipelines to unravel the intricate relationship between stress and cancer metastasis. However, it is important to note that this field is still in its early stages, and we anticipate a proliferation of similar approaches in the near future, further advancing our understanding and treatment of cancer.

Keywords: cancer; molecular biology; single-cell techniques; stress; metastasis

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1. Introduction

Cancer remains one of the most demanding challenges of modern medicine. Ordinary cells transform into malignancy by acquiring distinctive traits provided with evolutionary advantages, commonly recognized as the 'hallmarks' of cancer. These traits encompass resistance to apoptotic signals, autonomy from external growth cues, the ability to stimulate vascularization, the evasion of immune eradication, and the development of invasive properties facilitating the establishment of metastases in distant organs within a conducive microenvironment [1]. Notably, during this transformative process, pre-malignant or malignant foci may undergo elimination, enter a dormant state with slow progression, or evolve into a clinically evident demonstration.

Moreover, a pivotal aspect of cancer progression that underscores the formidable nature of malignancies is metastasis. This complex process involves the spread of cancer cells from the initial tumor site to distant organs, heralding the potential for widespread disease and increased mortality. As cancer cells acquire invasive properties, they navigate through the circulatory or lymphatic system, facilitating their migration to distant parts of the human tissues [2]. Once these cells migrate to a permissive microenvironment, they undergo a complex interaction with surrounding tissues to grow into secondary tumors, a phenomenon known as metastatic colonization. Metastasis is a critical turning point in cancer biology, complicating treatment efforts and significantly affecting patient outcomes. Understanding the underlying mechanisms leading to metastasis is crucial for developing targeted interventions to prevent cancer spread and enhance the effectiveness of therapeutic strategies.

Cancer treatment requires a versatile approach involving a range of therapeutic strategies aimed at targeting the disease at different levels [3]. Conventional therapies, such as surgery, chemotherapy, and radiotherapy, remain primary options, applied either alone or combined depending on the type and stage of the cancer [4]. Although these methods of treatment have shown efficiency in many cases, they are not universally proven to be successful due to various inherent limitations. First, the heterogeneity of cancer, even within the same type, is a significant challenge. A tumor can be presented with a variety of genetic mutations and cellular characteristics, which makes it difficult to devise a standard treatment approach. In addition, these therapies may cause irreversible damage to healthy surrounding tissues, leading to harmful side effects and restricting the optimal tolerated treatment capacity. Moreover, cancer cells can develop resistance to chemotherapy over time, rendering once effective drugs ineffective. The complexity of metastasis is another major concern, because conventional treatments may not adequately address widespread cancer cells, which may also be resistant to them [5].

Molecular biology is at the forefront of cancer research, providing valuable insights into the complex molecular and genetic cancer field [6]. Genomic profiling reveals specific genetic mutations and alterations within cancer cells, allowing for the classification of cancer types based on their unique molecular features. Moreover, biomarker discovery through molecular studies serves as a powerful tool for early cancer detection, assessing the prognosis, and monitoring the response to treatment [7]. Immunotherapy, a revolutionary approach to cancer treatment, owes its success to a deep understanding of the molecular interactions between cancer cells and the immune system [8]. Molecular biology also provides a deep insight into epigenetic modifications, revealing alterations in gene expression that can be targeted to reverse abnormal gene regulation in cancer cells [9]. These insights gained through molecular biology not only advance our knowledge of cancer but also lead to the development of new drugs and treatments, giving brand new opportunities to more precise, personalized, and targeted interventions in the continuous effort to beat this complex disease.

In the field of cancer research and treatment, big data approaches are a revolutionary force, enriching the molecular biology agenda with unprecedented insights and opportunities. Vast amounts of different types of computational data, including genomic profiles, clinical records, and imaging datasets, can be combined, providing an unprecedented view of a patient's clinical image. Big data analysis, capable of processing large and complex datasets, is becoming crucial for uncovering the unique genetic profiles of individual cells, delineating distinct cell populations, and distinguishing subtle variations in gene expression [10]. This approach, enhanced by big data, not only improves diagnostic accuracy but also pushes treatment decisions to be more personalized.

However, the precision of single-cell techniques increases the scope of molecular studies. These techniques, capable of analyzing heterogeneity within tissues and tumors at an unprecedented resolution, produce abundant molecular data [11]. The combination of big data and single-cell techniques not only improves our understanding of cellular heterogeneity but also enables more precise classifications of cancer subtypes and the identification of new therapeutic targets based on the specific molecular signatures of individual cells. In addition, single-cell analysis provides detail for studying the dynamics of gene expression, identifying variations in gene activity, and revealing regulatory networks.

While significant strides have been made in understanding the genetic and molecular foundations of cancer, the influence of stress on cancer progression, particularly metastasis, remains an area of active investigation in oncology. Stress, both physiological and psychological, is known to elicit complex physiological responses mediated by neuroendocrine pathways, including the release of stress hormones. These hormones, in turn, modulate various cellular processes implicated in cancer metastasis, including immune function, inflammation, and angiogenesis. Additionally, chronic stress has been shown to dysregulate key signaling pathways involved in cancer cell proliferation, invasion, and survival [3]. Despite growing evidence suggesting a potential link between stress and

cancer metastasis, elucidating the precise mechanisms underlying this relationship poses significant challenges due to the complex nature of stress responses and the heterogeneity of cancer. Nonetheless, ongoing research efforts leveraging advanced molecular techniques and integrative approaches hold promise for unraveling the intricate interplay between stress and cancer metastasis.

In this review, we aim to evaluate the current state of research on stress and cancer metastasis critically, identify gaps in knowledge, and outline paths for future research. By summarizing existing findings and addressing the challenges involved in studying this highly intricate relationship, we hope to elucidate the opportunities and challenges for understanding the impact of stress on cancer metastasis.

2. A Review of Molecular Biology, Single-Cell Techniques, and the Impact of Stress in Cancer Metastasis—Exploring Related Computational Studies and Future Frontiers

Recognizing the urgency to address the current state of molecular biology and single-cell techniques in the context of cancer research, this review begins a thorough examination of relevant studies, published documents, and recent research efforts. Our attention is directed towards elucidating the crucial role that stress plays in the complex process of cancer metastasis. Stress, in its various forms, has emerged as an important factor contributing to the progression and proliferation of cancer cells. By collecting and compiling the relevant literature, this review aims to shed light on the molecular mechanisms underlying the relationship between stress and cancer metastasis. Through this comprehensive analysis, the intention is to contribute valuable insights to the recently developing field, setting the stage for future research directions and potential therapeutic interventions targeting stress-mediated pathways in metastatic cancer.

2.1. Chemotherapy Response and Stress in Cancer Metastasis

It has been shown that single-cell RNA sequencing (scRNA-seq) data can reveal transcriptional patterns associated with chemotherapy resistance in high-grade serous ovarian cancer (HGSOC), particularly highlighting stress-promoted chemoresistance in metastatic cases [12]. Initially, they give some basic information about how HGSOC is characterized by high intratumor heterogeneity and TP53 mutations, platinum-based chemotherapy and its widespread usage in metastatic cancer treatment, and how platinum resistance is a common challenge in HGSOC, which would lead to poor survival rates. While poly (adenosine diphosphate–ribose) polymerase (PARP) inhibitors show potential for treating a type of ovarian cancer (HGSOC) that responds well to platinum-based drugs and has specific DNA repair issues, many patients without these DNA repair issues have fewer treatment choices when the cancer becomes resistant to chemotherapy. So, this research tries to explore how ovarian cancer cells respond at the genetic level to chemotherapy, using data from single-cell RNA sequencing and comparing samples from patients before and after receiving neoadjuvant chemotherapy.

In the scRNA-seq analysis of 11 homogeneously treated HGSOC patients, a sophisticated understanding of chemotherapy-induced processes emerged through the utilization of PRIMUS (Poisson scRNA integration of mixed unknown signals), a novel clustering approach. PRIMUS, designed to discern phenotypic cell groups while factoring in patient-specific variability, dataset-specific components, and technical noises, facilitated the identification of twelve distinct cancer cell clusters. These clusters were delineated by diverse gene signatures indicative of crucial biological processes such as differentiation, proliferation, DNA repair, epithelial-to-mesenchymal transition (EMT), and stress responses. Notably, the impact of chemotherapy on these clusters revealed a significant decline in proliferative DNA repair cells and an enrichment of stress-associated cells following neoadjuvant chemotherapy (NACT). Specifically, the stress-associated state, characterized by a core acute stress response, inflammatory signaling, and prosurvival mechanisms, exhibited subclonal enrichment during chemotherapy. Intriguingly, leveraging PRIMUS, it was observed that this stress-associated state independently predicted a poor prognosis

regardless of the homologous recombination deficiency status. PRIMUS's holistic approach, utilizing a bilinear Poisson regression model to simultaneously factorize expression data into defined nuisance factors, undefined cellular phenotypes, and their corresponding transcriptomic profiles, provided a comprehensive understanding of the intricate cellular dynamics underlying chemotherapy responses in HGSOC.

The research integrates human pancreatic datasets for comparison and evaluates the PRIMUS method against other integration techniques. Beyond this, the analysis employs differential expression analysis to identify stress-associated gene communities within cancer cells. These stress scores and proliferation scores are quantified and further validated using RNA in situ hybridization (RNA-ISH) and imaging techniques. Additionally, the study delves into the intricacies of cellular interactions and heterogeneity within the tumor microenvironment (TME). To elucidate cell-to-cell interactions, Nich-eNet analysis [13] is utilized, leveraging its computational approach to study intercellular communication. Nich-eNet harnesses human or mouse gene expression data of interacting cells, integrating this with a prior model that incorporates existing knowledge on ligand-to-target signaling paths. This predictive model enables the identification of potential ligand–receptor interactions that may drive gene expression changes in the cells of interest. Moreover, the trajectory analysis of stromal cells sheds light on their relationships within the TME. Broadening the scope, bulk tumor expression data, TCGA RNA-seq data, and TCGA reverse phase protein array data are leveraged for comprehensive validation and a deeper understanding of the clinical relevance of the findings.

Another recent study has demonstrated that the immunosuppression landscape induced by chronic unpredictable mild stress (CUMS) promotes colorectal cancer metastasis [14]. In their research, they delved into the intricate relationship between chronic stress-induced depression and colorectal cancer (CRC) progression. Utilizing single-cell RNA sequencing technology, the study aimed to unveil the molecular mechanisms involved. Given that chronic stress-induced depression significantly heightens the risk of metastasis and leads to an unfavorable prognosis in CRC, understanding this connection becomes pivotal. The study sheds light on the complex dynamics within the tumor immune microenvironment (TIME), providing valuable insights into potential targets for intervention and enhancing our comprehension of the factors influencing cancer outcomes.

Using single-cell RNA sequencing, researchers mapped the transcriptional landscape of the tumor immune microenvironment (TIME) in CRC-bearing mice under chronic stress. This method uncovered the genetic links between depression and CRC progression. They identified distinct myeloid cell subpopulations, including neutrophils and macrophages, with notable differences between the control and stressed groups. Chronic stress reduced anti-tumor neutrophil subpopulations, suggesting immunosuppressive effects. Macrophages in stressed mice shifted towards the M2 subtype, known for anti-inflammatory and tissue repair functions, contributing to an immunosuppressive environment that promotes CRC progression. This study enhances our understanding of immune dynamics in this context.

Another type of cell that this study pays attention to is T lymphocytes cells and their subsets, CD8+ T cells, effector T cells, and regulatory T cells (Tregs), which are a type of white blood cell essential for adaptive immune responses. Alterations in these cell types suggest a broader impact on adaptive immune responses. Gene expression changes within lymphocyte populations provide insights into shifts in immune function and inflammatory responses under chronic stress. Moreover, chronic stress induces a reduction in cell–cell interactions within the TIME, disrupting the information flow. This phenomenon has implications for immune evasion mechanisms and emphasizes the need for a holistic understanding of the immune microenvironment. CD8+ T cells in the chronic stress group exhibit a heightened expression of inhibitory immune checkpoints, contributing to immune suppression. To provide a potential explanation for the observed poor prognosis in CRC a pseudo-chronological analysis suggests that effector T cells may transform into exhausted T cells under chronic stress. The last subtype of T lymphocyte cells that this research

tries to explain is gamma delta T cells. This is a subtype of white blood cells that plays a crucial role in the immune system, and chronic stress appears to compromise their anti-tumor activities. The reduced proportions and altered gene expression profiles of these cells suggest that their ability to counteract tumor development may be hindered under conditions of chronic stress.

In this study, single-cell RNA sequencing (scRNA-seq) data were analyzed using the Seurat package for quality control, analysis, and exploration. We identified highly variable genes and performed graph-based clustering with the FindClusters function [15]. Cell populations were visualized with the UMAP algorithm [16], a dimensionality reduction technique. To assess pathway activities at the single-cell level, we used Gene Set Variation Analysis (GSVA) [17] and compared pathway activities between groups using the LIMMA software package [18]. For further insights, we conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses using Gene Set Enrichment Analysis (GSEA) with gene sets from the MSigDB database.

To delve into cell communication dynamics, we utilized the CellChat v1 R package [19], renowned for its proficiency in ligand–receptor interaction analysis. This involved importing a standardized expression matrix, transforming it into a CellChat object, preprocessing the data, and identifying potential ligand–receptor interactions. Meanwhile, to unravel the intricate cellular differentiation trajectories, pseudotime analysis was conducted using the Monocle2 package [20]. This process entailed converting Seurat objects into CellDataSet objects, filtering genes, performing dimensionality reduction clustering, and inferring cell differentiation trajectories.

Using cutting-edge methodologies like CellChat and Monocle2, we gained a holistic understanding of single-cell gene expression, clustering, pathway activities, intercellular communication, and cell differentiation trajectories. CellChat's Ligand–Receptor Interaction Explorer and Cell–Cell Communication Atlas Explorer enabled us to explore ligand–receptor interactions and cell–cell communications in our scRNA-seq data. Monocle2 provided insights into the developmental trajectories of cellular populations. This comprehensive approach unveils new aspects of cellular dynamics, offering valuable insights into the underlying biology of these populations. Despite advancements, cancer metastasis remains a primary challenge.

A recent study explores the intricate processes underlying cancer metastasis, with a particular focus on the role of hypoxia, hypoxia-inducible factor HIF-1, and angiogenesis [21]. The research tries to identify the connections between the hypoxic microenvironment, HIF-1 activation, and the promotion of metastatic phenotypes, especially in aggressive breast tumors. It highlights the pivotal role of angiogenesis, stimulated by hypoxia-regulated factors like vascular growth factor (VEGF-A), in tumor growth and metastasis. Additionally, the study investigates the impact of endoplasmic reticulum (ER) stress on cancer progression, highlighting the involvement of the unfolded protein response (UPR) and its downstream effectors.

The study employs a comprehensive approach, integrating molecular analyses and functional experiments. By targeting the protein disulfide oxidase endoplasmic oxidoreductin 1 alpha (ERO1), the researchers uncover its significant association with aggressive breast tumors. ERO1 is found to be highly expressed in basal breast cancer cells, particularly those associated with triple-negative breast cancer (TNBC), and its levels are further upregulated under hypoxic conditions. The research unveils that ERO1 ablation selectively impairs the migration of highly metastatic breast cancer cells without significantly affecting tumor growth. Intriguingly, the study highlights ERO1's specific impact on the oxidative protein folding of angiogenesis-related factors in hypoxic conditions, leading to a suppression of metastasis.

To explore breast cancer progression mechanisms, we utilized transcriptomics, focusing on Endoplasmic Reticulum Oxidoreductin-1 (ERO1) mRNA expression in breast cancer cell lines via RNAseq data from the Cancer Cell Line Encyclopedia (CCLE). Computational analysis of these genomic datasets revealed significant insights into breast cancer's

molecular landscape. To improve the analysis' accuracy, we developed PRISM, a statistical framework extracting sample composition and cell-type-specific whole-transcriptome profiles. Integrating transcriptomics with PRISM advanced our understanding of breast cancer pathogenesis, offering potential for improved diagnostic and therapeutic strategies.

2.2. Tumor Microenvironment and Cellular Interactions

The results indicate that breast cancer (BC) poses a formidable challenge, being a leading cause of cancer-related mortality in women [22]. Estrogen receptor-positive (ER-positive) breast cancer, characterized by cancer cells with receptors binding to estrogen, frequently evolves into bone metastasis (BM). Despite its clinical significance, the intricate molecular mechanisms underlying BM remain elusive, impeding progress in treatment strategies. A recent study reveals the relationship between tumor microenvironment cells and oxidative stress in breast cancer bone metastases. A global single-cell landscape atlas of BM was meticulously constructed using state-of-the-art scRNA-seq technology. The findings illuminated the dynamic interplay among diverse cell types, including BC cells, osteoblasts, mesenchymal stem cells (MSCs), immune cells, fibroblasts, and endothelial cells, collectively contributing to the complexity of BM. Particularly noteworthy was the reduction observed in BC cells within BM, while MSCs and fibroblasts exhibited significant abundance, underscoring their pivotal roles in BM development. Importantly, oxidative stress emerged as a substantial factor influencing the survival and progression of BC cells within the bone microenvironment.

In this study, the scRNA-seq analysis captured 16,409 high-quality single-cell profiles, delineating 43 distinct cell clusters within the BM TME. The identification of eight cell types and an imbalance in BC, MSC, and fibroblast populations in BM highlighted the intricate nature of BM progression. Increased oxidative stress levels in BM underscored its potential as a critical factor in BC cell apoptosis. The exploration of BC cell heterogeneity uncovered ten distinct subpopulations, each enriched in oxidative stress response and apoptosis-related pathways. Enrichment in cancer-related signaling pathways implicated these subpopulations in BM progression. Differentiation trajectories of BC cell subpopulations revealed specific transcription factors regulating their development and differentiation.

From these subpopulations, eight fibroblasts exhibited significant abundance in BM, linking them to processes of ossification, bone remodeling, and cancer-related pathways. Fibroblast subpopulations followed distinct differentiation trajectories and were regulated by specific transcription factors, emphasizing their role in BM. Another ten Mesenchymal Stem Cells (MSC) were associated with Bone Morphogenetic Protein (BMP) signaling, which is crucial for bone development and regeneration, and Tumor Necrosis Factor (TNF), which is a cytokine involved in various cellular processes including inflammation and immune responses, skeletal growth, and oxidative stress responses. The differentiation trajectories of MSC subpopulations unveiled their regulatory mechanisms, pointing towards potential clinical implications. Finally, the analysis of intercellular communication highlighted strong interactions between fibroblasts and BC cells in controls, while BM samples exhibited increased BC cell interactions with various cell types.

In this study, single-cell RNA sequencing (scRNA-seq) data pertinent to bone metastasis (BM) in breast cancer were acquired from the Gene Expression Omnibus (GEO). To construct a comprehensive single-cell atlas, the scRNA-seq data were merged using the IntegrateData function of the Seurat package in the R language. Subsequent to cell clustering analysis, the results were visualized using the uniform manifold approximation and projection (UMAP) technique, offering a two-dimensional representation termed a single-cell atlas. Cell types were annotated based on known markers derived from prior studies. Differential gene expression analysis was conducted to identify genes that exhibited significant changes between single cells of primary tumor tissue and BM tissues. The "FindAllMarkers" function of the Seurat package was employed for this purpose, with a significance threshold set at adjusted p-values < 0.05 and a $|\log \text{fold change (logFC)}| > 0.5$.

To unravel the intricate molecular mechanisms underlying dysregulated genes, we conducted functional enrichment analyses, specifically focusing on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Leveraging the clusterProfiler R package [23], we employed a significance threshold of $p < 0.05$ to discern biologically relevant processes and pathways associated with the dysregulated genes. Furthermore, we evaluated oxidative stress-related pathways by employing the Seurat package's AddModuleScore function, providing insights into the impact of oxidative stress on cellular processes. In addition, we delved into the developmental trajectory of dysregulated cells in both primary tumor tissues and bone marrow (BM) tissues through pseudo-time analysis. This analysis, conducted using the Monocle 3 package in R, enabled the gaining of a deeper understanding of the temporal dynamics and progression of dysregulated cells. The resulting trajectories were visualized through Uniform Manifold Approximation and Projection (UMAP), facilitating the interpretation of complex cellular behaviors. The comprehensive functionality of these analytical tools, including clusterProfiler for efficient functional annotation and Monocle 3 for scalable and robust single-cell analysis, allowed for deciphering the intricate molecular landscapes associated with dysregulated genes. By integrating functional enrichment analyses and pseudo-time analysis, this study gained valuable insights into the biological processes and pathways driving dysregulation in primary tumor and BM tissues, thereby advancing our understanding of cancer pathogenesis.

To delve deep into the regulatory mechanisms governing cellular processes, gene regulatory network (GRN) analysis was conducted using the Python module pySCENIC [24]. This advanced pipeline facilitated the reconstruction of transcription factor-centered gene regulatory networks, providing insights into the intricate web of regulatory interactions within the cell. Regulator activity scores and specificity scores were quantified to elucidate the regulatory dynamics driving gene expression. The resulting networks were rigorously validated and visually inspected to discern relationships and hierarchies, shedding light on the complex regulatory landscape. Additionally, to explore cellular communication events between different subpopulations of cells, this study employed high-confidence ligand–receptor interactions identified using the iTALK package in R [25]. This powerful toolkit, developed by the Linghua Wang lab at the University of Texas MD Anderson Cancer Center, offers a comprehensive platform for characterizing and visualizing intercellular communication dynamics. By leveraging iTALK, they were able to gain insights into the complexity, diversity, and dynamics of cell–cell communication across various biological processes.

It is worth mentioning the impact of the method and conditions of tumor dissociation on the outcomes of single-cell RNA sequencing, focusing on the utilization of collagenase at different temperatures. The overarching goal is to comprehend the effects of the dissociation process on the cell yield, transcriptome states, and stress responses across a diverse range of samples, including patient cancer tissues, patient-derived breast cancer xenografts (PDXs), and cancer cell lines. Notable observations include the significant variability of key quality control metrics related to cell viability across different dissociation conditions and tissues. These metrics encompass the total number of genes detected, the percentage of transcripts mapping to the mitochondrial genome, and the total number of unique molecular identifiers (UMIs) sequenced [26].

A crucial finding is the identification of a stress response induced by collagenase digestion at 37 °C, which led to the identification of a set of 512 heat shock and stress response genes, including important genes such as FOS and JUN. Interestingly, this stress response is attenuated when a cold-active protease is used at 6 °C. The study highlights the conservation of this transcriptional pattern associated with collagenase in different cell types and tissues, indicating a common cellular response to this particular dissociation method.

The study highlights the profound impact of the chosen tumor dissociation method on both the cell performance and transcriptome status, potentially introducing artifacts that could complicate the interpretation of scRNA-seq data. It sheds light on subpopulations of dead and dying cells distinguished by their mitochondrial gene content, warning of their

potential impact on immune recognition analysis. Furthermore, a comparative analysis between collagenase and a cold-active protease suggests the second as a promising alternative method for scRNA-seq experiments with tumor tissues, as it appears to minimize the observed stress response.

This study employed a robust approach to differential expression analysis, harnessing the power of the edgeR tool [27] to investigate the transcriptional response to heat stress across diverse biological contexts, including patient-derived xenografts and cell lines. Leveraging the quasi-likelihood F test, recognized as a top-performing method in recent evaluations, we rigorously assessed differential gene expression while accounting for both technical and biological variations. To ensure robustness, genes with a minimum count of 10 across all cells were considered, and the core set of heat-responsive genes was defined based on stringent criteria: an FDR-adjusted Q value < 0.05 and a $|\log2(\text{fold change})| > \log2(1.5)$. This stringent criterion identified 192 genes, with 182 upregulated and 10 downregulated genes. Furthermore, to gain insights into the biological processes affected by heat stress, pathway enrichment analysis was conducted using the camera tool on the Hallmark gene set [28]. This approach allowed for uncovering enriched pathways associated with the heat stress response, providing a deeper understanding of the molecular mechanisms underlying cellular adaptation to thermal stress. Additionally, to elucidate temporal changes induced by heat stress, pairwise comparisons were conducted over specific time intervals, enabling the tracking of digestion enzyme-induced alterations over time. This meticulous analysis strategy provided insights into the dynamic nature of the transcriptional response to heat stress, shedding light on the temporal dynamics of gene expression changes.

Cell type assignments in this study were conducted using the CellAssign algorithm [29], a sophisticated probabilistic model that integrates both pre-defined and de novo cell types based on known marker genes. By leveraging marker genes, CellAssign probabilistically annotated cell types, offering flexibility in marker expression levels across different cell types. This approach facilitated accurate and robust cell type assignments, even in the presence of variations in marker expression levels. To delve deeper into cellular dynamics in response to heat stress, the hierarchical clustering of live, dying, and dead cells was performed using the 10-dimensional output of Matching Neighborhood Networks (MNN). The resulting clusters were assigned using the cutree function [30], revealing distinct patterns of cell behavior in response to heat stress. This analytical approach allowed for the identification and characterization of different cellular states and their responses to environmental stimuli.

Glioblastoma (GBM) is a highly aggressive primary intracranial cancer with limited treatment success and a poor prognosis. Immunotherapy has shown promise in cancer treatment, but GBM's unique challenges include the "immune privilege" of the brain and the immunosuppressive microenvironment within GBM. Macrophages, comprising a significant portion of infiltrating immune cells in GBM, play a crucial role in tumor occurrence and progression [31]. This study employed single-cell analysis, with standardized data using R software, which revealed distinct clusters, with macrophages significant in the tumor core. Differences in the cell composition and marker genes were observed between the tumor core and peritumoral tissue. The macrophages in the GBM displayed increased oxidative stress activity, suggesting their involvement in the tumor's stress response.

Macrophages were elevated in the tumor core. Transcription factors related to macrophage pathways were identified. Differential gene analysis highlighted significant pathways, e.g., arthritis in macrophages and apelin signaling in microglia. The macrophages exhibited high interaction intensity, particularly with microglia. Specific receptor–ligand pairs were identified. The trajectory analysis showed macrophages at the center, indicating a central role in interactions with other cell subsets. Gene modules associated with macrophages (M1 and M3) were identified through WGCNA. Two MR-DEGs, MANBA (high-risk) and TCF12 (low-risk), were identified. The model demonstrated good predictive performance with distinct survival outcomes. M1 macrophages were negatively correlated with risk scores and MANBA, suggesting MANBA's potential role in immune responses. GO and KEGG analyses revealed

pathways related to the extracellular matrix, chemokine signaling, and immune responses. MANBA knockdown in glioblastoma cell lines resulted in reduced proliferation, migration, and invasion, supporting its role in GBM progression.

To ensure the reliability of downstream analyses, a rigorous quality control step was conducted, leveraging the DoubletFinder R package, which predicts doublets in single-cell RNA sequencing data. This step helped exclude capsule cells and filter out low-quality cells, thus enhancing the accuracy of subsequent analyses. Additionally, to assess the activities of cellular oxidative stress pathways, the AUCell R package was utilized, enabling the identification of cells with active gene sets in single-cell RNA-seq data. This method employs the "Area Under the Curve" (AUC) to determine whether a critical subset of the input gene set is enriched within the expressed genes for each cell, offering a robust approach independent of gene expression units and normalization procedures.

Moreover, to identify macrophage-associated transcription factor (TF) regulatory networks exhibiting significant changes in proportion, the pyscenic method was employed. This involved utilizing GRNBoost, a scalable strategy for gene regulatory network (GRN) inference built on top of Apache Spark. Inspired by GENIE3, GRNBoost breaks down the inference problem into tree-based ensemble (Random Forest) nonlinear regressions, constructing predictive models for the expression profile of each gene in the dataset based on the expression profiles of candidate regulatory genes (transcription factors). By reframing the GENIE3 approach into an Apache Spark MapReduce-style pipeline and utilizing Gradient Boosting, GRNBoost improves the runtime performance and scalability, facilitating the inference of large-scale GRNs from high-throughput gene profiling data. Additionally, the potential interaction strength of macrophages with other cells was predicted based on the mean expression number of receptors and ligands using the cellchat R package.

2.3. Computational Approaches and Methodological Insights

A recent study closely related to our area of interest reveals the mechanisms of ovarian cancer, how it often spreads to the peritoneal cavity, leading to the formation of ascites fluid and tumor growth in the abdominal area, and the role of tumor-associated macrophages (TAMs) in the peritoneal tumor environment and their influence in cancer progression [32]. Accounting for over 50% of the cells in peritoneal tumor implants and fluid ascites in ovarian cancer patients, TAMs, unlike their counterparts which maintain normal tissues, can support tumor growth while suppressing immune responses. Efforts to target TAMs for cancer immunotherapy have faced challenges; however, a successful transfer to clinical applications has been prevented. This study explores the complex landscape of peritoneal resident macrophages in ovarian cancer models by identifying two distinct subsets of TAMs through the use of the Tim-4 marker: Tim-4+ and Tim-4− TAMs. The characterization of these subsets reveals that Tim-4+ TAMs align with specific gene profiles and functions, suggesting their embryonic origin as resident macrophages. In contrast, Tim-4− TAMs display links to monocyte-derived macrophages, exhibiting distinct characteristics.

The further investigation of TAM subgroups reveals an increased expression of specific markers, such as GATA6, in Tim-4+ TAMs, indicating an association with long-term residential macrophages. Furthermore, the transcriptional profiles of Tim-4+ TAMs differ significantly, suggesting functional differences. The ontogeny of Tim-4+ TAMs suggests an embryonic origin and local self-expansion within the tumor microenvironment, contributing substantially to tumor growth and making them potential targets for therapeutic intervention. Metabolically, Tim-4+ TAMs exhibit increased mitochondrial activity and participate in mitophagy to eliminate damaged mitochondria, a process that becomes particularly important in the context of oxidative stress. Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) and antioxidants, is implicated in affecting TAM function. Arginase-1, an enzyme crucial for mitochondrial fitness and the regulation of mitophagy in Tim-4+ TAMs, is identified as a potential factor affected by oxidative stress, thereby influencing the functionality of TAMs in the tumor microenvironment.

The deficiency of autophagy emerges as a decisive factor leading to the loss of Tim-4+ TAMs, possibly due to increased mitochondrial damage, oxidative stress, and increased ROS production. The consequences of this deficiency extend to tumor progression, resulting in slowed growth and enhanced T cell-mediated antineoplastic immune responses. The relevance to human ovarian cancer patients is highlighted through the identification of human counterparts of Tim-4+ TAMs labeled by CRIg. In summary, this study provides a comprehensive investigation of TAM subgroups in ovarian cancer, shedding light on their characteristics, ontogeny, and potential as therapeutic targets.

In this study [33], a comprehensive analysis of RNA-Seq data was conducted using the Tuxedo Suite software package, which offers a range of tools for various RNA-Seq analyses, including alignment, differential expression analysis, and post-analysis diagnostics. Initially, reads were aligned to the reference transcriptome (hg19) using TopHat (version 2.0.13), a fast splice junction mapper for RNA-Seq reads, followed by a thorough quality control assessment post-alignment. Subsequently, Cufflinks/CuffDiff (version 2.2.1) was utilized to quantify expression levels, normalize data, and perform differential expression analysis. Diagnostic plots were generated and analyzed using the cummeRbund R package [34] to assess the quality of the analysis results. For enrichment and gene ontology analysis, the Gene Set Enrichment Analysis (GSEA) software from the Broad Institute was employed, which computed enrichment scores and simulated enrichment scores for each variable and signature. Furthermore, to explore Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and gene ontology terms in human tumor-associated macrophages (TAMs), the Enrichr web server [35] was utilized, which provided a comprehensive analysis and allowed for the identification and prioritization of important KEGG pathways and gene ontology terms within the context of the study.

In the realm of colorectal cancer (CRC) metastasis and its underlying molecular mechanisms, we cannot overlook the potential of lycorine, a phenanthridine alkaloid found in the Amaryllidaceae family [36]. Lycopene has previously been identified as a compound with a variety of biological activities, and it has anti-cancer activity. The methodology employed in this study includes the use of RNA-seq analysis to examine the changes in gene expression profiles in CRC cells treated with lycorine. The results reveal a significant alteration in the transcriptome, with a notable upregulation of genes related to apoptosis and a downregulation of genes associated with cell proliferation. These findings suggest that lycorine induces programmed cell death in CRC cells, potentially inhibiting their uncontrolled growth.

One key aspect explored in the research is the activation of the endoplasmic reticulum (ER) stress pathway by lycorine. The endoplasmic reticulum is a cellular organelle crucial for protein folding, and disturbances in its function lead to ER stress. The study identifies the involvement of ER stress-related genes, such as DDIT3 and EIF2S1, in the response to lycorine treatment. This activation of the ER stress pathway is proposed as a mechanism through which lycorine exerts its anti-cancer effects. Furthermore, the impact of lycorine on various signaling pathways, including the TNF (tumor necrosis factor) signaling pathway, has been examined. The modulation of genes within this pathway suggests a link between lycorine treatment and the regulation of apoptosis. The study emphasizes the complexity of the molecular interactions involved and highlights the potential of lycorine as a therapeutic agent against CRC. It is evident that lycorine may inhibit the growth of colorectal cancer cells. The identified pathways, such as ER stress and TNF signaling, contribute to our understanding of lycorine's anti-cancer properties at the cellular level.

The experimental approach in this study encompasses the utilization of RNA-seq (RNA sequencing) analysis, which allows for a comprehensive examination of the transcriptome within colorectal cancer (CRC) cells subjected to lycorine treatment. This methodology enables the identification of alterations in gene expression patterns, offering an understanding of the molecular responses induced by lycorine. The observed changes in the transcriptome point towards a significant impact on key cellular processes. Notably, lycorine treatment results in the upregulation of genes associated with apoptosis, the programmed cell death

mechanism crucial for maintaining tissue homeostasis. Concurrently, a downregulation of genes linked to cell proliferation is observed, suggesting a potential hindrance to the uncontrolled growth characteristic of cancer cells.

An interesting aspect of the study involves the investigation of the stress pathway in the endoplasmic reticulum (ER). Through the activation of ER stress-related genes, such as DDIT3 and EIF2S1, lycopene appears to induce stress within the endoplasmic reticulum. This cellular organelle is crucial for proper protein folding, and perturbations in its function may lead to the activation of signaling pathways involved in cell survival and apoptosis. The identification of ER stress in responses to lycorin sheds light on a potential mechanistic pathway through which the compound exerts its anticancer effects. In a similar way, the modulation of the TNF (tumor necrosis factor) signaling pathway by lycorine plays a crucial role in regulating cellular responses to stress and inflammation, and its involvement in lycorine-induced changes indicates a complex interplay between the compound and apoptotic regulation.

The latest developments in cancer have given rise to a new wave of possibilities, as the role of Zinc Finger DHHC-Type Containing 1 (ZDHHC1) seems to be a tumor suppressor [37]. ZDHHC1 is frequently silenced in tumors due to promoter methylation. ZDHHC1 has the ability to inhibit cancer cell proliferation, induce apoptosis, and suppress migration and invasion. Zinc Finger DHHC-Type Containing 1 (ZDHHC1) has the potential to regulate glucose metabolism pathways and its connection with the Cytoglobin (CYGB) protein. ZDHHC1 has a great impact on reactive oxygen species (ROS), ER stress, and the induction of pyroptosis and necroptosis in cancer cells. As a consequence, it contributes to cellular stress, leading to programmed cell death mechanisms. This study proposes that targeting ZDHHC1-mediated metabolic signaling pathways, including its effects on oxidative stress, ER stress, and cell death mechanisms, may be a promising strategy for cancer prevention and therapy.

The study employed iTRAQ proteomics and GC-MS metabolomics to investigate the molecular intricacies of the experimental conditions. iTRAQ proteomics provided insights into protein expression patterns, focusing on dynamic changes associated with ZDHHC1 and its impact on cellular pathways. GC-MS metabolomics assessed cellular metabolite profiles, revealing metabolic alterations induced by ZDHHC1 modulation. Shotgun proteomics identified and characterized proteins, while iTRAQ facilitated simultaneous identification and quantification for high-throughput analysis. In GC-MS metabolomics, samples were ionized using electron ionization (EI) for stable spectra, aiding in compound identification via comparison with spectral libraries. Chemical ionization (CI) was also utilized for enhanced compound identification.

In addition, rigorous statistical analyses were conducted using the SPSS software platform, which encompasses a wide range of statistical tests including Student's T test, the chi-square test, and Fisher's exact test. These analyses were essential for determining the statistical significance of the observed results, ensuring the reliability of the findings. The application of robust statistical evaluation added a layer of confidence to the study's conclusions, reinforcing the validity of the experimental outcomes and their implications for understanding ZDHHC1's role in the investigated cellular processes.

2.4. Therapeutic Targets and Molecular Insights

The significance of statins, known for their cholesterol-lowering properties, cannot be ignored due to their anti-cancer effects [38]. Statins exert their anti-cancer effects by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), a key enzyme in the mevalonate pathway. Mechanistically, statins disrupt cellular signaling pathways crucial for cancer cell survival by depleting mevalonate-derived isoprene metabolites. Despite these insights, the intricate molecular factors determining statin sensitivity and the consequent impact on cancer cell homeostasis remain unclear.

The study revealed significant differences in gene expression and metabolite profiles between statin-sensitive and statin-resistant cell lines. Statin treatment induced pro-

nounced changes in statin-sensitive HOP-92 cells, impacting cell cycle-related pathways and DNA replication. In contrast, statin-resistant NCI-H322M cells exhibited fewer alterations. Metabolomic analyses highlighted distinct responses to atorvastatin, emphasizing changes in glycolytic intermediates, central carbon metabolism, purine metabolism, and polyamine levels. Statin-sensitive cells exhibited reduced glucose uptake, inhibited glycolysis, and altered polyamine levels, indicating vulnerability to statin-induced oxidative cellular stress.

The observed differences in molecular responses underscored the complex interplay between statins and cancer cell metabolism. The study implicated statin-induced oxidative stress and altered redox states as potential contributors to statin sensitivity, suggesting a link between metabolic vulnerabilities and the efficacy of statin treatment. Moreover, the study uncovered potential implications for cancer metastasis. Statin-sensitive cells displayed altered cytoskeletal structures and a decreased expression of genes related to the Rho GTPase effector pathway, indicating a potential hindrance in metastatic capabilities. The findings were consistent with the notion that statins may contribute to metastasis suppression through oxidative stress in statin-sensitive cancer cells.

The total RNA extracted from atorvastatin-treated cells underwent comprehensive analysis through RNA sequencing using an Illumina NovaSeq 6000 sequencer. The resulting data were subjected to various analytical techniques, including principal component analysis (PCA), partial least squares regression (PLS), and hierarchical clustering. PCA, a dimensionality reduction method, simplified the large dataset while retaining significant patterns and trends, providing insights into the transcriptional landscape. PLS regression, a technique combining features from PCA and multiple regression, was employed to predict dependent variables from a large set of independent variables, enhancing the analysis' predictive power. Hierarchical clustering grouped metabolites extracted from cells using capillary electrophoresis time-of-flight mass spectrometry and CE-tandem mass spectrometry, facilitating the identification and quantification of metabolites. This method created clusters in a dendrogram, visually representing similarities between metabolites. The data obtained from various experiments were statistically evaluated using BellCurve for Excel, incorporating the Student's *t*-test and two-way analysis of variance with the Tukey–Kramer post-hoc test. The significance level was set at $p < 0.05$, ensuring a robust statistical foundation for the study's findings and facilitating the identification of treatment- or factor-level differences through post hoc analysis with Tukey's test.

The intricate relationship between oxidative stress, adherens junctions, and cancer metastasis, particularly in hepatocellular carcinoma (HCC), has been under consideration [39]. The study highlights the dual role of reactive oxygen species (ROS) in cancer progression and metastasis. Adherens junctions, crucial for tissue morphogenesis, are identified as key players in preventing cancer cell metastasis, with the loss of E-cadherin (ECAD) representing an early event in metastasis. The conflicting studies on ROS's impact on metastasis are addressed, emphasizing the need for a comprehensive understanding. The research unveils a novel mechanism by which oxidative stress induces ECAD protein degradation through the RING finger protein 25 (RNF25), identified as a novel E3 ligase of ECAD ubiquitination. Mechanistic insights reveal that protein kinase A (PKA) undergoes redox modification in response to ROS, leading to RNF25 phosphorylation and subsequent ECAD protein degradation. The study employs in vitro and in vivo models, clinical sample analyses, and molecular assays to substantiate the critical role of the ROS-PKA-RNF25-ECAD axis in driving HCC metastasis. Furthermore, the findings suggest potential therapeutic targets, such as RNF25, for impeding metastasis and improving patient outcomes in HCC.

The empirical findings support the proposed mechanism. Experimental evidence demonstrates that oxidative stress promotes HCC metastasis in an orthotopic mouse model, and high nitrotyrosine levels correlate with an advanced stage and poor survival in HCC patients. H_2O_2-induced oxidative stress is shown to repress the ECAD protein at the post-translational level, independent of transcriptional regulation. RNF25 is identified

as a crucial player in this process, acting as an E3 ligase for ECAD ubiquitination. The phosphorylation of RNF25 at Ser450 is pinpointed as a key event in initiating ECAD protein degradation. The study further elucidates the involvement of PKA, with the catalytic subunit beta (PRKACB) acting as a redox sensor that responds to oxidative stress and phosphorylates RNF25. The dynamic regulation of redox signaling and ECAD expression in different stages of tumor metastasis is discussed, revealing a potential dual role of the PKA/RNF25/ECAD pathway in both early EMT and later metastatic stages. Clinically, a high expression of RNF25 is associated with HCC metastasis and poor prognosis, establishing the translational relevance of the proposed mechanism.

The experimental section delves into exploring the impact of oxidative stress on cancer metastasis through a diverse array of methods. Liver cancer cell lines were cultured and manipulated to elucidate the role of RNF25. Utilizing in vivo models with mice, the effects of treatments such as NAC and Oltipraz were investigated. The analysis of clinical HCC tissues provided valuable insights, supplemented by various assays including Transwell and co-immunoprecipitation, which probed cellular responses. Additionally, a comprehensive molecular investigation was conducted, employing mass spectrometry, protein kinase activity assays, ubiquitination assays, and phosphorylation assays to uncover the underlying molecular mechanisms driving the observed phenomena. To assess the significance of the findings, a range of statistical analyses including *t*-tests, Analysis of Variance (ANOVA), Pearson correlation tests, and Kaplan–Meier analyses were applied. T-tests were employed to determine significant differences between the means of two groups, while ANOVA was utilized to compare variances across the means of different groups. The Pearson correlation coefficient was computed to measure the linear correlation strength and direction between variables. Finally, the Kaplan–Meier estimate facilitated survival probability computation over time despite associated complexities, offering valuable insights into the experimental outcomes.

Breast cancer remains a major health concern for women worldwide, with recurrence and metastasis being primary causes of treatment failure. A recent study investigated the role of Ring finger protein 126 (RNF126), an E3 ubiquitin ligase, in breast cancer metastasis and its potential as a target for Ataxia telangiectasia mutated and Rad3-related kinase (ATR) inhibitors [40]. An analysis of gene expression datasets reveals a positive association between RNF126 expression and breast cancer metastasis. Additionally, RNF126 is found to be upregulated in breast tumor tissues compared to normal tissues. Weighted gene coexpression network analysis (WGCNA) identifies cell cycle pathways enriched in patients overexpressing RNF126.

Functional experiments demonstrate that RNF126 promotes breast cancer cell proliferation, growth, migration, and invasion in vitro and in vivo. ATR inhibitors are shown to be more effective in killing breast cancer cells with intact RNF126 compared to cells with RNF126 knockdown. This increased sensitivity is associated with elevated replication stress in cells expressing higher levels of RNF126. Mechanistically, RNF126 knockdown reduces replication stress, making breast cancer cells less sensitive to ATR inhibitors. Moreover, CDK2 is identified as a mediator of ATR inhibitor-induced cell death in breast cancer cells with high RNF126 expression.

In this study, a comprehensive analysis was undertaken utilizing a variety of methods, including the application of bioinformatics tools to uncover metastasis-related genes and signaling pathways implicated in breast cancer progression. Initially, Weighted Gene Co-expression Network Analysis (WGCNA) was employed to identify clusters of highly correlated genes from microarray data. This facilitated the identification of metastasis-related genes and provided insights into their functional roles. Subsequently, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to elucidate the functional and pathway enrichment of these identified genes, providing a deeper understanding of their biological significance. Additionally, Gene Set Enrichment Analysis (GSEA) was utilized to further investigate the differential expression of genes associated with metastasis, allowing for a more nuanced exploration of gene expression patterns.

The analysis was complemented by the use of the ClusterProfiler package in R, which enabled comprehensive functional enrichment analysis across diverse genomic datasets. Furthermore, ImageJ software was employed for the quantification of DNA fibers in DNA fiber assays, facilitating a comprehensive understanding of DNA replication dynamics and its potential implications in cancer metastasis. Overall, these approaches provided valuable insights into the molecular mechanisms underlying breast cancer progression and metastasis.

2.5. Stress Response and Molecular Mechanisms

In the rapidly evolving field of cancer research, the role of glutathione peroxidase 2 (GPX2) in cervical cancer progression has recently garnered attention. This study underscores the pressing need for molecular markers in cervical cancer diagnosis and treatment strategies [41]. While GPX2, an antioxidant enzyme, has been implicated in various cancers, its specific role in cervical cancer remains ambiguous. Previous research suggests that GPX2 may contribute to cancer progression by regulating hydroperoxide levels and key pathways such as epithelial-to-mesenchymal transition (EMT) and WNT/β-catenin signaling. To elucidate its role in cervical cancer, the study utilized clinical specimens of cervical cancer tissues and cell lines for in vitro experiments. The techniques employed included quantitative real-time PCR (qRT-PCR), Western blotting, transient transfection, immunoblot analysis, the measurement of reactive oxygen species (ROS), colony formation assays, migration, invasion assays, and immunohistochemistry. Notably, qRT-PCR, a pivotal advancement in PCR technology, enabled the reliable detection and measurement of products generated during each cycle of the PCR process. This technique relies on the introduction of an oligonucleotide probe designed to hybridize within the target sequence.

The study's findings have shown a significant impact of stress, particularly oxidative stress, on cancer dynamics. Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and cellular detoxification mechanisms, emerges as a pivotal determinant of cancer progression. Elevated oxidative stress levels can instigate cellular damage, fueling tumor growth, invasion, and metastasis. Notably, GPX2's role in modulating oxidative stress levels within cancer cells emerges as a critical focal point. By influencing ROS levels and subsequent cellular responses, GPX2 exerts a significant influence on cancer cell behavior, including proliferation, migration, and invasion.

A new target gene, HSPA6, has recently been identified in triple-negative breast cancer (TNBC) cells treated with thymoquinone (TQ), a key component of black seed oil known for its anti-cancer properties [42]. HSPA6, encoding a 70 kDa heat shock protein, was found to be significantly upregulated by TQ treatment and exhibited inhibitory effects on TNBC cell growth, migration, and invasion, which has implications for metastatic potential. Given that heat shock proteins are involved in cellular responses to stress, particularly heat and oxidative stress, this finding suggests a potential link between stress response pathways and breast cancer biology.

The study further explored the regulatory mechanisms, prognosis, and clinical significance of HSPA6 in breast cancer. Notably, a high expression of HSPA6 was positively correlated with longer overall survival in patients with both subtypes of breast cancer and TNBC, suggesting a potential tumor-suppressive role for HSPA6. Mechanistically, the research investigated the methylation status of the HSPA6 promoter region in breast cancer tissues, revealing that DNA methylation may not be the primary regulatory mechanism for HSPA6 mRNA upregulation. Additionally, the study explored the interplay between TQ and HSPA6, demonstrating that TQ enhanced the inhibitory effects of HSPA6 overexpression on migration and invasion while attenuating the effects when HSPA6 was knocked down.

After conducting the quality control and preprocessing of RNA-seq data using Trimmomatic software, which performs a variety of useful trimming tasks for Illumina paired-end and single-ended data, the clean reads were aligned using the STAR software. Gene expression levels were then quantified by counting reads mapped to the exon regions of each

gene using featureCounts software, a highly efficient read summarization program. Subsequently, normalization was performed to obtain Reads Per Kilobase per Million mapped reads (RPKM) values. The edgeR package was employed for the subsequent differential gene expression analysis, with a statistical significance cutoff of $p < 0.05$ and a fold-change cutoff of 2.

To elucidate the biological functions and pathways associated with the differentially expressed genes, Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were conducted. The KOBAS software, which annotates sequences with KEGG Orthology terms and identifies significantly enriched pathways, was utilized for pathway analysis, with a significance threshold of $p < 0.05$. Additionally, to validate the expression changes of selected genes identified through bioinformatics analysis, semi-quantitative RT-PCR and western blot assays were employed. Semi-quantitative PCR provided a rapid method for estimating the relative number of messages in RNA populations, using a known housekeeping gene as an internal standard to normalize the expression levels of the target gene of interest.

In order to improve our understanding of non-small cell lung cancer (NSCLC) and its function, the intricate relationship between stress hormones and resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs) has to be explored [43]. This study posits that stress hormones like norepinephrine (NE) and epinephrine (E) activate β2-adrenergic receptors (β2-ARs) on NSCLC cells, leading to an increased expression of interleukin-6 (IL-6), a known mediator of EGFR TKI resistance. Through a series of in vitro and in vivo experiments using EGFR mutant NSCLC cell lines and animal models, the study demonstrates that the stress hormone activation of β2-ARs promotes resistance to EGFR TKIs. Furthermore, it elucidates the underlying molecular mechanisms, revealing that β-AR signaling inactivates the tumor suppressor liver kinase B1 (LKB1), which contributes to therapeutic resistance and metastasis in NSCLC. The clinical relevance of these findings is underscored by analyses of patient data, showing that high IL-6 levels are associated with poorer outcomes in EGFR TKI-treated patients, while β-blocker use is linked to lower IL-6 levels and a potentially improved response to EGFR inhibitors.

The implications of this study are several. First, it gives us valuable knowledge about a new mechanism by which stress hormones drive therapeutic resistance in NSCLC, providing insights into the tumor microenvironment's role in treatment responses. Additionally, the findings suggest a promising therapeutic strategy involving the repurposing of β-blockers, which are widely available and well-tolerated drugs, in combination with EGFR-targeting agents to overcome resistance. The study's proposal for clinical trials testing this combinatorial approach represents a significant step towards addressing the challenge of EGFR TKI resistance in NSCLC patients. Overall, by elucidating the complex interplay between chronic psychological stress, tumor biology, and treatment responses, this study opens avenues for further research and underscores the potential for innovative therapeutic interventions in NSCLC management.

Several bioinformatics methodologies were employed to analyze and interpret the experimental data. Gene expression analysis and sequencing were conducted to examine gene expression patterns and sequences, with subsequent bioinformatics tools utilized for data processing and interpretation. Statistical and computational analyses were performed for various aspects of the study, including in vitro experiments, gene expression profiling, and clinical analyses, utilizing bioinformatics methods for data preprocessing, normalization, and statistical testing. Reverse Phase Protein Array (RPPA) studies were conducted to quantify protein expression levels, with bioinformatics tools utilized for data analysis, including normalization, quality control, and statistical analysis. RPPA is a high-throughput antibody-based technique, similar to Western blots, where hundreds to thousands of different cell lysates are immobilized on a nitrocellulose-coated slide as many individual spots. These spots undergo incubations with protein-specific antibodies for detection. Antibodies are organized into sets, typically comprising several hundred,

and are used for each assay. The antibody sets may be adjusted based on feasibility or functionality, forming new sets as needed.

As it is shown in Figure 1, this review tries to comprehensively analyze several studies related to stress and cancer metastasis. Initially, the most studies explore how stress impacts cancer metastasis, setting the stage for further discussion. These studies then try to show the link between these by using molecular biology and various data types. Following this, the computational tools that have been employed in this research area are examined, such as machine learning algorithms, gene regulatory network (GRN) analysis, and statistical methods, which are crucial for data interpretation and validation. Concluding, the key mechanisms that link stress and cancer metastasis are illustrated, offering a comprehensive overview of the current methodologies used to investigate this complex relationship. Additionally, Table 1 presents a comprehensive summary of each study included in the review. The table details the population studied in each study, the interventions and the comparisons made, the outcomes and the bioinformatics tools used. This synopsis facilitates a thorough understanding of the research landscape and highlights the methodologies and tools employed across different studies.

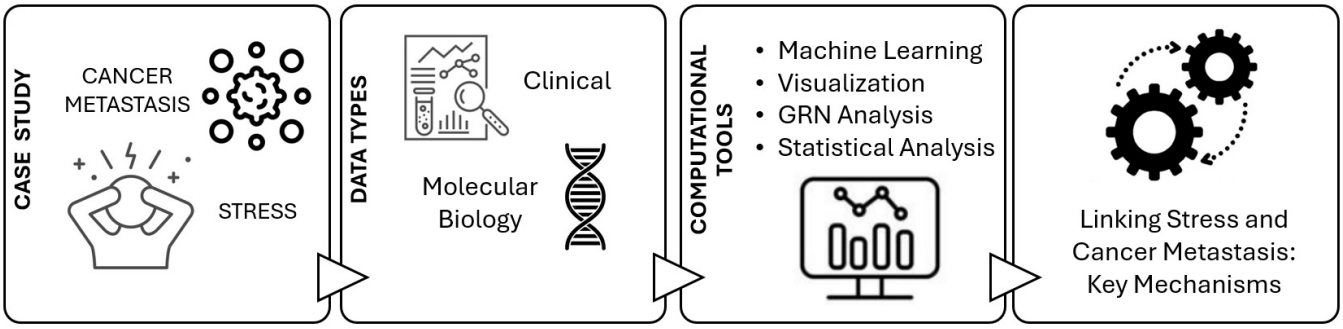

Figure 1. Overview of the studies reviewed.

Table 1. Summary Table of the Studies.

Study	Population	Intervention	Comparison	Outcomes	Bioinformatics Tools
[12]	Eleven high-grade serous ovarian cancer (HGSOC) patients undergoing platinum-based chemotherapy	Response of ovarian cancer cells at the genetic level to chemotherapy, using data from single-cell RNA sequencing linked to the stress-associated state	Patients before and after chemotherapy	Stress-Associated Enrichment in Chemotherapy Predicts Poor Prognosis; Chronic Stress Links to CRC Progression and Metastasis; TME Analysis Identifies Therapeutic Targets in Breast Cancer Bone Metastases	- PRIMUS - NichNet
[14]	Human CRC cell line CT26,40 BALB/c mice	Chronic stress-induced depression and colorectal cancer (CRC) progression using single-cell RNA sequencing	CUMS-induced depression CRC tumor-bearing mice and control group	Chronic Stress Induced Depression's Role in CRC Progression; Uncovering Molecular Mechanisms; Stress Links to Metastasis and Poor CRC Prognosis	- Seurat - UMAP - GSVA - LIMMA - GSEA - CellChat - Monocle2
[21]	Breast cancer patients	Analysis of ERO1 levels	Breast tumors; normal breast tissue; aggressive cancer cells	Impaired migration in ERO1 KO cells; Decreased breast tumor growth; Higher ERO1 expression in basal breast tumors; Higher risk of tumor recurrence; Inverse correlation between ERO1 levels and time to first metastases	- Prism 7
[22]	Breast cancer patients with bone metastases	Single-cell RNA sequencing technology for constructing a global single-cell landscape atlas of bone metastasis (BM)	Control and bone metastasis samples	Holistic TME View: Therapeutic Targets in Breast Cancer Bone Metastases; Interplay of Cell Types and Oxidative Stress in BM Development	- Seurat - UMAP - GO & KEGG - clusterProfiler - AddModuleScore - Monocle3 - pySCENIC - iTALK
[26]	A total of 48 samples and 155,165 cells from patient cancer tissues, patient-derived breast cancer xenografts (PDXs), and cancer cell lines	Utilization of collagenase at different temperatures (37 °C and 6 °C) and comparison with a cold active protease at 6 °C	Collagenase dissociation at 37 °C and 6 °C and dissociation using a cold active protease at 6 °C	Cell Yield Evaluation and Transcriptome State Assessment; Stress Response Identification and Quality Control Metrics Analysis	- edgeR - camera tool - CellAssign - MNN - Cutree function

Table 1. *Cont.*

Study	Population	Intervention	Comparison	Outcomes	Bioinformatics Tools
[31]	GBM Patients	Macrophages in GBM core and peritumoral tissue; Oxidative stress activity analysis	Tumor core and peritumoral tissue; macrophages and different cell types; high- and low-risk groups	Differences in macrophages and other cells; Increased oxidative stress activity in macrophages; High interaction types and intensity in macrophages; Identification of MANBA and TCF12 as prognostic genes	- DoubletFinder - AUCell - PercentageFeatureSet - AddModuleScore - GRNBoost - cellchat
[32]	Ovarian cancer patients with peritoneal tumor implants and ascites fluids	Studying two types of TAMs in peritoneal macrophages. Analyzing gene profiles, functions, and metabolic features including mitochondrial activity and mitophagy.	Tim-4+ and Tim-4− TAM subsets; tumor growth and immune responses in the presence and absence of Tim-4+ TAMs	Tim-4+ TAMs in Tumor Microenvironment: Ontogeny and Functional Role; Metabolic Characteristics and Implications for Therapeutic Interventions; Autophagy Deficiency Effects on Tumor Progression and Immune Responses; Human Counterparts Identification for Ovarian Cancer Relevance	- TuxedoSuite - TopHat - Cufflinks/CuffDiff - cummRbund - GSEA - EnrichR
[36]	CRC Cells (HCT8, CT-26, HCT116, HT-29), Mice	Treatment with lycorine hydrochloride	No treatment (Control), Mitoxantrone (Positive control)	Assessing cell viability, colony formation, wound healing, invasion, apoptosis, protein expression, RNA-seq, real-time PCR, lung metastasis, and histological staining	- RNA-seq
[37]	Cancer patients; Nude mice in xenograft model	Genetic modulation of ZDHHC1 expression	ZDHHC1 expression in normal-tumor tissues; Control vector-transfected cells	ZDHHC1 curbs cell proliferation, prompts apoptosis, and hinders migration, invasion, and tumor growth, reversing EMT and stemness and regulating metabolism	- iTRAQ proteomics - GC-MS metabolomics - SPSS software
[38]	Human epithelial NCI-H322M and mesenchymal HOP-92 lung cancer-derived cell lines	The therapeutic potential of statins for inhibiting cancer proliferation.	Cell lines before and after atorvastatin treatment	Baseline disparities between epithelial (NCI-H322M) and mesenchymal (HOP-92) lung cancer cells; Atorvastatin affects transcriptome more in HOP-92	- PCA - PLS regression - Hierarchical clustering - BellCurve - Tukey–Kramer post-hoc test
[39]	HCC patients	The role of Oxidative Stress in Liver Cancer Metastasis	Metastatic and non-metastatic samples	Oxidative stress activates PRKACB, phosphorylating RNF25, leading to ECAD degradation, and promoting HCC metastasis; potential therapeutic implications identified	- *t*-tests - ANOVA - Pearson correlation tests - Kaplan–Meier analyses

Table 1. *Cont.*

Study	Population	Intervention	Comparison	Outcomes	Bioinformatics Tools
[40]	Breast cancer patients	Knockdown of RNF126 in breast cancer cells and treatment with ATR inhibitors	Control (cells without RNF126 knockdown) and cells with RNF126 knockdown	Cell proliferation, growth, migration, and invasion; sensitivity to ATR inhibitors, apoptosis, replication stress, tumor growth inhibition, and metastases reduction	- WGCNA - GO - KEGG - GSEA - ClusterProfiler
[41]	Cervical cancer patients	Examination of GPX2 expression in cervical cancer cells and tissues	GPX2 expression levels between cervical cancer tissues and normal tissues and between different stages of cervical cancer	Increased GPX2 was correlated with cervical cancer progression, metastasis, EMT/WNT activation, ROS regulation, apoptosis inhibition, and pathological stage association	- qRT-PCR
[42]	Breast cancer patients	HSPA6 expression and TQ treatment	Normal tissues and breast cancer tissues	TQ treatment enhanced HSPA6-mediated inhibition of cell migration and invasion; knocking down HSPA6 attenuated TQ effects	- Trimmomatic software - STAR software - featureCounts software - edgeR package - GO - KEGG - KOBAS - Semi-quantitative RT-PCR - Western blot assays
[43]	NSCLC patients	Potential of treatment employing EGFR tyrosine kinase inhibitors (TKIs), stress hormones, β-blockers, and EGFR-targeting agents	No treatment or treatment with inhibitors	Increased IL-6 expression was associated with EGFR TKI resistance; β-blocker use was linked to lower IL-6 levels and improved survival	- RPPA

3. Clinical Implications

The computational findings presented in these studies have profound clinical implications across various areas of cancer research. They have identified molecular pathways associated with chemotherapy resistance in ovarian cancer and colorectal cancer, as well as potential therapeutic targets such as stress-associated states and specific immune cell populations. Understanding these pathways could lead to the development of innovative treatment strategies that target resistant cancer cells or modulate the tumor microenvironment to enhance treatment efficacy. Additionally, by analyzing single-cell RNA sequencing data, researchers can identify molecular signatures associated with drug sensitivity or resistance in individual patients. This information can be used to tailor treatment regimens based on the unique genetic profile of each patient, leading to more effective and personalized therapeutic approaches.

Computational analyses have revealed potential biomarkers, such as ZDHHC1 in cancer metabolism pathways or GPX2 in cervical cancer progression. These biomarkers could serve as diagnostic tools for early cancer detection, prognostic indicators for predicting patient outcomes, or predictive markers for selecting appropriate treatment options. The identification of specific molecular targets, such as RNF126 in breast cancer metastasis or HSPA6 in triple-negative breast cancer, provides opportunities for the development of targeted therapies aimed at inhibiting key drivers of cancer progression. These targeted therapies could offer more effective and less toxic treatment options for patients with advanced or metastatic disease.

The findings have also elucidated mechanisms of resistance to various cancer treatments, including EGFR TKIs in non-small cell lung cancer. By understanding these resistance mechanisms, researchers can develop strategies for overcoming treatment resistance and improving patient outcomes. The insights gained can inform the design of clinical trials by identifying patient populations most likely to benefit from specific treatments or combinations of treatments. This can lead to more efficient and successful clinical trials, accelerating the translation of research findings into clinical practice. By identifying factors associated with cancer progression, such as oxidative stress or ER stress pathways, risk assessment and prevention strategies can be developed, including lifestyle modifications or targeted interventions aimed at reducing the risk of cancer development or progression.

4. Summary

The reviewed articles collectively indicated the intricate relationship between stress and cancer metastasis across various cancer types. Chronic stress emerges as a significant factor influencing cancer progression and metastasis, particularly evident in colorectal cancer (CRC) and breast cancer bone metastases. Stress-associated enrichment in chemotherapy predicts poor prognosis in CRC, while chronic stress-induced depression exacerbates CRC progression by unraveling molecular mechanisms that link stress to metastasis and unfavorable prognoses. Moreover, an integral view of the tumor microenvironment (TME) reveals the interplay of stress-induced oxidative stress, cellular interactions, and therapeutic targets in promoting breast cancer bone metastases. Stress not only impacts tumor cells directly but also influences the tumor microenvironment, affecting the behavior of immune cells like TAMs (Tumor-Associated Macrophages) and their functional role in tumor progression and metastasis. Additionally, stress-induced oxidative stress activates pathways like PRKACB, leading to the degradation of ECAD and promoting metastasis in hepatocellular carcinoma (HCC). The findings underscore the importance of considering stress as a critical factor in cancer progression and metastasis, suggesting potential therapeutic implications targeting stress pathways to mitigate metastatic spread and improve patient outcomes.

Current computational approaches in cancer research, especially those employing AI models, encounter significant limitations. One key challenge is the potential biases within AI models stemming from biases in the training data, leading to algorithmic biases. Interpreting single-cell RNA sequencing (scRNA-seq) data is also challenging due to the complexity and heterogeneity of cancer cells, along with the variability in sample

preparation and experimental conditions. Additionally, the dynamic nature of cancer progression requires integrating multi-omics data and longitudinal studies, posing further computational and analytical challenges. Addressing these limitations is vital for ensuring the reliability of findings and advancing our understanding of cancer biology to improve patient outcomes.

5. Discussion

Cancer is one of the most daunting challenges in modern medicine. The transformation of normal cells into malignant entities is marked by the acquisition of distinct characteristics, collectively known as the 'hallmarks of cancer'. These include resistance to apoptotic signals, autonomy from external growth cues, the ability to stimulate vascularization, the evasion of immune surveillance, and invasive properties that enable metastasis in permissive microenvironments. This transformational process sees pre-malignant or malignant foci either being eliminated, entering a dormant state, or progressing to clinically evident diseases.

Recent studies underscore the significant role of chronic stress in cancer progression. Stress has been shown to promote tumor growth and progression through immunosuppressive effects and complex interactions between tumor cells and their microenvironment [44]. Notably, stress hormones such as norepinephrine and cortisol are implicated in enhancing the invasive potential of ovarian cancer cells, likely via the stimulation of matrix metalloproteinases (MMPs) [45]. Another pivotal factor in cancer progression is mechanical stress within the tumor microenvironment. High mechanical stress can profoundly alter cancer cell metabolism and behavior, contributing to tumor progression and metastasis [46]. This understanding is crucial for developing strategies aimed at modulating the tumor microenvironment.

Furthermore, chronic stress has been observed to activate neural-inflammatory signaling pathways, which remodel the lymphatic vasculature and increase the lymph flow. These changes provide enhanced pathways for tumor cell dissemination, thereby accelerating metastasis [47]. This insight offers new therapeutic targets for managing stress-induced physiological changes and limiting metastasis. The advent of single-cell technologies has dramatically advanced our understanding of tumor heterogeneity, metastasis, and drug resistance [48]. These technologies allow for the detailed profiling of individual cells within tumors, revealing unique genetic and molecular pathways that are pivotal in cancer progression.

The role of oxidative stress in cancer metastasis is also gaining attention—particularly, its capability to limit the spread of human melanoma cells [49]. This suggests that modulating oxidative stress could be a viable therapeutic strategy for controlling cancer spread. Additionally, the neuroendocrine system, influenced by psychological stress, plays a significant role in cancer development and metastasis. Interventions that target neuroendocrine pathways, such as enhancing endogenous β-endorphin levels, have shown promise in reducing stress responses and lowering cancer incidence in prostate and breast cancer models [22].

Considering these findings, the challenges in translating complex data from single-cell technologies into clinical practice cannot be overstated. These advancements highlight the need for personalized treatment approaches that consider the intricate interplay of genetic, environmental, and individual patient factors in cancer progression. However, integrating such detailed molecular insights into clinical decision-making remains a significant challenge. Future research should focus on bridging these gaps, paving the way for more effective, targeted diagnostic tools and therapies. Ultimately, these developments in understanding cancer at a molecular level hold the promise of significantly impacting future cancer diagnostics, treatment, and prevention strategies, steering us towards more adaptive and personalized cancer care.

6. Conclusions

Our comprehensive exploration of cancer's multifaceted nature underscores the profound impact of chronic stress on tumor growth and progression. As we have unraveled, stress not only manipulates the tumor microenvironment through hormonal and inflammatory pathways but also influences immune surveillance and angiogenic processes. This has been exemplified in our findings, particularly highlighting how stress hormones like norepinephrine and cortisol can augment the invasive potential of cancer cells. Intriguingly, the innovative use of single-cell technologies has offered an unprecedented glimpse into the heterogeneity of cancer at a molecular level, revolutionizing our approach towards personalized and targeted cancer therapies. However, the challenge lies in translating these complex molecular insights into tangible clinical applications, a task that calls for continued research and innovation.

Looking ahead, it is evident that combating cancer necessitates a holistic approach. This approach must integrate molecular and cellular understandings with broader physiological and environmental considerations. The insights from our study not only enrich the existing knowledge pool in oncology but also chart promising pathways for future research and treatment strategies. Our collective aim remains steadfast—to enhance patient outcomes and advance the global fight against this formidable disease, all while navigating the intricate interplay of biological, environmental, and stress-related factors in cancer progression.

The further elucidation of stress-associated pathways and their impact on tumor development and therapy responses across different cancer types has to be carried out. This could involve comprehensive studies using advanced technologies like sc-RNA seq to dissect the transcriptional landscapes of tumor microenvironments under stress conditions, such as chronic stress or chemotherapy-induced stress. Additionally, there is a need to explore the potential of targeting stress response pathways as therapeutic strategies to enhance treatment efficacy and overcome resistance. Furthermore, future research should aim to develop more precise and effective methods for tumor dissociation in single-cell RNA sequencing experiments to minimize artifacts and improve data quality. Integrative approaches combining multi-omics analyses, functional experiments, and clinical data could provide comprehensive insights into the molecular mechanisms driving cancer progression and guide the development of novel therapeutic interventions. Moreover, investigating the role of stress hormones and their receptors in tumor biology and treatment resistance, as well as exploring the therapeutic potential of targeting stress signaling pathways, could open new avenues for personalized cancer therapy.

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