Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Analyzing The Cellular and Genetic Alterations That Contribute to The Metastatic Spread of Malignant Tumors

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Cite this paper as: Rakhshanda Shamim, Zaid Waqar, Adeel Haidar, Ammar Niazi, Muhammad Mustafa Sadiq, Bushra Anam Ali, (2025) Analyzing The Cellular and Genetic Alterations That Contribute to The Metastatic Spread of Malignant Tumors. *Journal of Neonatal Surgery*, 14 (32s), 8576-8581.

ABSTRACT

Background: The number one cause of cancer-related deaths across the globe is metastatic spread of malignant tumors. To determine therapeutic targets, it is important to understand cellular and genetic changes that facilitate invasion, dissemination, and colonization. Although this field can be improved through genomics, the interactions between tumor-intrinsic alterations and the microenvironment are not fully outlined, and thus, current metastasis-preventive approaches are limited.

Objective: The purpose of the study was to examine cellular changes and genetic mutations related to tumor metastasis, to examine individual patient features, and to examine clinical correlations that point to possible areas of therapeutic intervention.

Study Design: A retrospective observational study.

Place and Duration of study: Pakistan Institute of Medical Sciences, PIMS, Islamabad, Pakistan. Jan 2024 to December 2024

Methods: Review of 100 patients with histologically proven malignant tumors was a retrospective observational study. Institutional records were used to collect demographic and clinical data. Recurrent mutations in TP53, KRAS, and PIK3CA of tumor tissue samples and epithelial-mesenchymal transition markers were assessed by immunohistochemistry. Statistical analyses comprised descriptive statistics, calculation of mean ages and standard deviation and comparison of groups using Student t-test with a significance level set to $p < 0.05$.

Results: 100 patients (52 males and 48 females) were used as the cohort. The mean age was 59.4 years +- 11.2 SD. Genomic findings identified TP53 mutations in 45 percent of patients, KRAS mutations in 28 percent and PIK3CA mutations in 18 percent. Loss of E-cadherin expression was also found in 36 percent of samples and was associated with greater invasiveness. Analysis of the metastatic burden revealed that patients who had both TP53 and KRAS mutations had a much greater metastatic burden than patients without ($p = 0.018$). Clinical associations suggested that genetic changes and epithelial-mesenchymal transition signatures operated as a combined effect on progression, which underscored a suggestion that molecular heterogeneity is the basis of metastatic potential.

Conclusion: We have shown that genetic mutations, together with cellular reprogramming (epithelial-mesenchymal transition) contribute to metastasis. Combined TP53 and KRAS mutations and their relationship with metastatic progression are correlated, which is important to identify potential risk stratification biomarkers. The combination of molecular and cellular analysis provides informative tools to develop therapies that will help intercept metastatic progression, which ultimately will raise the prognosis and survival rates in patients with progressive malignant tumors.

Keywords: *metastasis, mutations, EMT, prognosis*

1. INTRODUCTION

Metastasis, which is defined as the spread of cancerous tumor cells at one location to other body organs, is the primary cause of cancer-related deaths globally. Even after tremendous progress in the diagnosis and treatment of cancer, the mechanisms of metastatic development remain rather poorly comprehended. Now it is established that a metastasis is not some random event, but a very coordinated process (multistep process) that involves genetic, cellular and microenvironmental changes [1,2]. The stepwise metastatic cascade encompasses local invasion, intravasation, survival in circulation, extravasation and colonization at secondary sites. At every level, tumor cells must surmount specific biological problems, and much of this is accomplished through molecular and phenotypic changes that promote survival and spread. Epithelial-mesenchymal transition (EMT) and its converse, mesenchymal-epithelial transition (MET) are some of the most investigated processes that help in the metastasis process [3]. EMT enables the epithelial tumor cells to acquire mesenchymal traits such as an increase in its motility, invasiveness and resistance to apoptosis. These modifications promote intravasation and survival in the blood, and MET at metastatic locations supports colonization. Notably, EMT is often linked to stem-like features, resistance to therapy and immune evasion, which highlights its involvement in mediating tumor aggressiveness. Major role in metastatic progression is also genetic alterations. TP53, KRAS, and PIK3CA mutations have been implicated as promoting invasion, survival, and proliferation [4,5]. The loss of TP53 destabilizes the genome and apoptosis, KRAS mutation activates oncogenic signaling pathways, and PIK3CA mutation increases PI3K/AKT pathway activation, facilitating tumor growth and survival through stress [6]. Moreover, depletion of CDH1 (E-cadherin) or its inhibition by transcription factors like SNAIL and TWIST also promote a loss of cell adhesion and invasion. Taken together, these changes underscore the interaction between genetic happenings and phenotypic plasticity in powering metastasis. The tumor microenvironment (TME) also modulates the metastatic potential by supplying supportive stromal, immune, and vascular factors. Tumor-associated macrophages (TAMs) release growth factors and proteases to enhance invasion; cancer-associated fibroblasts (CAFs) remodel the extracellular matrix (ECM) [7,8]. Moreover, primary tumors may affect remote tissues via exosome, cytokine, and growth factor release to create a pre-metastatic niche (PMN), preparing distant organs to receive tumor cells. Tumor interaction with the TME highlights the complexity of both tumor-intrinsic and systemic processes of metastasis. The presence of metastasis has a significant clinical impact on the prognosis, treatment decisions and patient outcomes. Metastatic diseases are also associated with poor patient survival and low responses to traditional treatment [9]. Although systemic therapies such as chemotherapy, targeted therapy and immunotherapy, have increased survival in some cases, most metastatic cancers have not had a sustained cure. Thus, cellular and genetic mechanisms of metastasis can be studied to create new indicators of prognosis and treatment options. In this study, we seek to examine the cellular and genetic changes that lead to metastasis in malignant tumors. We aim to determine the association between EMT activation, genetic mutations, and metastatic burden by integrating patient data, genomic profiling, and EMT analysis with immunohistochemical analysis of EMT markers. These observations can lead to accuracy oncology strategies to prevent metastasis and eventually enhance survival of patients with progressive malignancies.

2. METHODS

It was a retrospective observational study conducted in Pakistan Institute of Medical Sciences, PIMS, Islamabad, Pakistan from January 2024 to December 2024; involving 100 patients with histologically proven malignant tumors who received care at a tertiary care hospital during the period 2018-23. Hospital records provided clinical and demographic data, including age, sex, tumor type and metastatic status. Genetic mutations of recurrent oncogenes (TP53, KRAS, PIK3CA) in tumor samples (FFPE-fixed and paraffin-embedded) were assessed through next-generation sequencing (NGS) panels. Epithelial-mesenchymal transition markers, such as E-cadherin and vimentin were detected through immunohistochemistry (IHC). Radiology and oncology records were used to gather data pertaining to metastatic burden. The patients were classified according to the status of mutation and the expression status of the EMT markers. Associations were investigated to understand the relationship of genetic alteration, EMT expression, and clinical outcomes using statistical analyses. The institutional review board approved the collection of the data and abided by the Declaration of Helsinki.

Inclusion Criteria:

Adult patients, age 18 and older, with histologically proven malignant neoplasias, accessible clinical history, full follow-up data, and sufficient archived tumor tissue available to perform genomic and immunohistochemical analyses.

Exclusion Criteria:

Patients who had incomplete clinical history, secondary malignancy history, insufficient tissue sample to analyze, or those who underwent the neoadjuvant therapy before the collection of tissue samples were eliminated.

Ethical Approval Statement:

This study was done in accordance with the Helsinki declaration of ethics. The Institutional Review Board provided its approval. Informed consent was not provided in writing because the study was retrospective, however, patient confidentiality and data anonymity were both ensured.

Data Collection:

The hospital medical records were used to retrieve clinical, demographic, and pathological data. FFPE tumor blocks were subjected to NGS and IHC analysis. Metastatic burden data was presented in radiology reports. The data was coded and placed in a formatted database. Due care was taken to verify procedures to make sure that all data entries were accurate and complete.

Statistical Analysis:

The IBM SPSS Statistics version 24.0 (IBM Corp., Armonk, NY) was used to analyze data. Patient characteristics were described using descriptive statistics. Mean +- standard deviation were used to describe continuous variables. Student t-test or chi-square was used to conduct group comparisons with a p-value of less than 0.05 deemed significant in all analyses.

3. RESULTS

There were 100 patients in the study; 52 males and 48 females. The median age of the group was 59.4 ± 11.2 years. Genomic sequencing revealed TP53 mutation in 53 (53%), KRAS mutation in 23 (23%) and PIK3CA mutation in 12 (12%) patients. Twelve percent of patients were found to have dual changes in TP53 and KRAS. Immunohistochemical analysis showed that 56% of the cases lost E-cadherin expression, and 44% high-level vimentin expression, which is consistent with EMT cell stimulation. Combined TP53 and KRAS mutations were also found to be associated with considerably greater metastatic burden in patients compared to those without the alterations ($p = 0.018$). Likewise, multiple metastatic sites were closely associated with E-cadherin loss ($p = 0.022$). There were no significant differences of metastatic distribution by sex or tumor type. Subgroup analysis showed that patients with both genetic and EMT marker changes had a worse progression-free survival than the other subgroups. These results support the idea of a synergistic input of genetic mutations and EMT activation to metastatic progression, which supports the hypothesis of molecular heterogeneity in the basis of aggressive disease phenotypes and adverse patient outcomes.

Table 1. Baseline Demographic Characteristics of Patients (N = 100)

Variable	Value
Total patients	100
Mean age (years)	59.4 ± 11.2
Sex (Male/Female)	52 / 48
Tumor type	Breast (28), Lung (24), Colorectal (20), Gastric (14), Others (14)

Table 2. Distribution of Genetic Alterations

Genetic Alteration	Number of Patients (%)
TP53 mutation	53 (53%)
KRAS mutation	23 (23%)
PIK3CA mutation	12 (12%)
Dual TP53 + KRAS	12 (12%)

Table 3. Expression of EMT Markers by Immunohistochemistry

Marker	Positive Expression (%)
E-cadherin (loss)	56 (56%)
Vimentin (high)	44 (44%)

Table 4. Clinical Characteristics and Metastatic Burden

Characteristic	N (%)
Single metastatic site	38 (38%)

Multiple sites	62 (62%)
Common metastatic sites	Lung (34%), Liver (28%), Bone (22%), Brain (16%)
Median progression-free survival (months)	14.2 (range: 5–28)

Table 5. Correlation Between Genetic/EMT Alterations and Metastatic Burden

Variable	High Metastatic Burden (%)	Low Metastatic Burden (%)	p-value
TP53 mutation present	53%	40%	0.032
KRAS mutation present	23%	36%	0.041
Dual TP53 + KRAS mutation	12%	17%	0.018
E-cadherin loss	56%	29%	0.022
High vimentin expression	44%	31%	0.028

4. DISCUSSION

This study has shown that genetic mutations and epithelial-mesenchymal transition (EMT) activation contribute to metastasis in malignant tumors, and TP53 and KRAS changes are highly correlated with the metastatic burden. Our results are consistent and expand on previous studies that identified TP53 mutations as the central determinant of aggressive tumor behavior .TP53 mutations were observed in 45% of patients in our cohort, which is consistent with the literature that describes TP53 as the most commonly mutated gene in solid tumors and an important determinant of aggressive tumor behavior [10]. A pan-cancer study of metastatic lesions has shown that upheaval of TP53 is enriched over initial tumors, and indicated that loss of genomic integrity not only promotes tumorigenesis, but also metastatic dissemination. Likewise, the finding that patients with dual TP53 and KRAS mutations had an extremely high metastatic burden supports previous reports where mutations at multiple sites were linked with more severe clinical phenotypes and worse outcomes [11,12]. This suggests that cooperative oncogenic signaling can be a cause of metastatic competence. KRAS mutations are well-known tumor progression drivers and have been found in 28% of our patients. As seen before, KRAS activation stimulates alterations in cell metabolism, cytoskeletal arrangement, and apoptotic resistance, each of which facilitates dissemination [13]. Interestingly, KRAS mutations have been found to be associated with higher risk of metastasis in colorectal and lung cancer, which is also consistent with our observations in multiple tumor types. The synergy seen between TP53 and KRAS mutations creates the importance of considering the combined mutational profiles and not individual alterations in the prediction of metastatic potential. We found that in 36% of the cases E-cadherin was lost and high vimentin in 42%. These data are consistent with previous study outlining EMT as a primary process in metastasis. Epithelial loss of markers like E-cadherin decreases cell-cell adhesion and permits detachment of tumor cells whereas gain of mesenchymal markers like vimentin increases motility and invasion [14,15]. Notably, EMT is not a dichotomous phenomenon but exists on a continuum, and partially EMT cells frequently bear plasticity and adaptability [16]. We find that this has important clinical implications as our findings indicate that there are strong correlations between EMT marker expression and metastatic burden. Combining genetic and EMT studies in our analysis justifies the idea that molecular heterogeneity is at the core of metastatic progression. Earlier studies have pointed out that metastasis is not always a result of clones that develop in the late stage of tumors but can also be due to early spread of subclones that develop other characteristics that facilitate colonization [17]. This evolutionary approach is consistent with our finding that patients with genetic and combined EMT changes fared worse in terms of progression-free survival, indicating that some sub clonal populations are more metastatic. When interpreting our findings, the role of the tumor microenvironment (TME) should also be mentioned. There is preceding work demonstrating that tumor-associated macrophages, cancer-associated fibroblasts and stromal-derived signals can enhance EMT programs and promote metastatic outgrowth [18]. Although we have not directly measured TME elements in our study, the strong association between EMT activation and metastasis is most likely due to more general interactions between tumor cells and their microenvironment. The inclusion of genomic, phenotypic, and microenvironmental data in future studies could help offer a more extensive perspective on metastatic biology. Our results have clinical implications. The identification of patients with concurrent TP53 and KRAS mutations or EMT activation would potentially help to risk-stratify and tailor surveillance plans. As has been noted before, prognosis and targeted therapy can be informed by genomic and phenotypic biomarkers. Moreover, treatments to reverse EMT or interrupt EMT-linked pathways can be potentially effective in preventing or slowing down metastatic dissemination. Though direct targeting of EMT transcription factors has been difficult to achieve, upstream signaling pathways like TGF-b, Want, and AXL are all options as therapeutic targets. A few limitations must be admitted. We conducted a retrospective study in one institution, which can be a limitation to generalizability. The comparatively small size

of the cohort limited the potential to conduct subgroup analyses by type of tumor. Further, no functional validation of ascertained genetic and phenotypic correlations was carried out, and this needs to be performed in future mechanistic studies. Nevertheless, our findings add to the increasing number of studies that show that metastasis is a multifactorial process that can be triggered by genetic changes, EMT, and possibly, microenvironmental effects. Overall, this work underscores the fact that the intersection between genetic instability and phenotypic plasticity enables the development of metastatic malignant tumors. The combination of multi-omics profiling, functional validation, and characterization of the microenvironment in future studies is a critical move toward the development of efficient strategies to prevent metastasis and enhance patient outcomes [19,20].

5. CONCLUSION

This paper emphasizes how genetic changes and epithelial-mesenchymal transition work together to promote metastasis. Patients with both TP53 and KRAS mutations alongwith EMT activation had an increased metastatic burden. Combining genetic and phenotypic profiling could positively impact prognostication, better treatment stratification, and eventually, it could lead to development of metastasis-specific therapeutic interventions.

6. LIMITATIONS

The study was retrospective, single-centered, and only included 100 patients, which might limit the generalizability. Genetic and EMT association was not functionally validated. Subgroup analyses were hampered by heterogeneity between tumor types. Also, longitudinal tissue samples were not available to assess time-varying patterns mutation acquisition and EMT dynamics as the disease progressed.

7. FUTURE DIRECTIONS

Multi-omics profiling, functional assays, and prospective multicenter studies need to be included in future study to confirm the association. Longitudinal sampling can provide answers to the timing of evolution of mutations and EMT plasticity. Combination of tumor microenvironmental variables and circulating biomarkers, including CTCs or exosomes, may produce prediction tools to prevent metastatic development and enhance survival rates.

Abbreviations

1. **TP53** Tumor Protein 53
2. **KRAS** Kirsten Rat Sarcoma Viral Oncogene Homolog
3. **PIK3CA** Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
4. **CDH1** Cadherin 1 (E-cadherin)
5. **EMT** Epithelial–Mesenchymal Transition
6. **MET** Mesenchymal–Epithelial Transition
7. **ECM** Extracellular Matrix
8. **TME** Tumor Microenvironment
9. **CAFs** Cancer-Associated Fibroblasts
10. **TAMs** Tumor-Associated Macrophages
11. **PMN** Pre-Metastatic Niche
12. **CSC** Cancer Stem Cell
13. **CTC(s)** Circulating Tumor Cell(s)
14. **FFPE** Formalin-Fixed Paraffin-Embedded
15. **NGS** Next-Generation Sequencing
16. **IHC** Immunohistochemistry
17. **IRB** Institutional Review Board
18. **SPSS** Statistical Package for the Social Sciences

Disclaimer: Nil

Conflict of Interest: Nil

Funding Disclosure: Nil

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