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Microbial Signatures and Molecular Pathology in Cancer Progression and Targeted Therapy

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ABSTRACT

The relationships between microbial communities and host biology also affect cancer progression and can significantly impact treatment effectiveness. This paper examines the association between microbial signatures and molecular pathology in cancer development and responses to targeted therapy. The participants were 150 cancer patients of different types of cancers, such as breast, colorectal, and lung cancer. The study was a prospective cohort study. The profile microbials were done by 16S rRNA sequencing, and molecular pathology was done by the evaluation of gene expression and mutational profiling of tumour samples. The main results were the identification of microbial taxa with reference to specific molecular subtypes and response to treatment. The researchers concluded that the presence of certain microbial communities had a major effect on the progression of tumors and on resistance to targeted therapy. It is important to note that Firmicutes and Bacteroides were associated with a higher response rate to targeted colorectal cancer therapy ($p < 0.05$). On the other hand, high concentration of Proteobacteria had a negative response among patients of breast cancer undergoing HER2-targeted treatments ($p < 0.01$). Such results indicate that microbial signatures have the ability to become potential biomarkers to forecast response to treatment and tumor progression. The paper highlights why microbial profiling should be included in the diagnosis and choice of therapy to enhance the development of personalized medicine approaches. These findings still need to be confirmed through further research, and the mechanisms that underlie the process of microbial control over cancer pathways need to be investigated.

Keywords: *microbial signatures, cancer progression, molecular pathology, metagenomics, targeted therapy, cancer biomarkers*

INTRODUCTION

Cancer is a complex disease that is caused by interactions between genetic changes and the tumor microenvironment (TME). In the recent past, the discovery of the essential functions of the microbiota in cancer progression, immune modulation, and therapeutic response has been made. The human microbiome

(bacteria, fungi, viruses, and archaea) is in contact with host cells and tissues, regulating inflammation and immune system activities, and even tumor formation [1]. Research has demonstrated that some microbial groups in the TME may or may not stimulate tumor growth based on their effects on oncogenesis and therapeutic response. Indicatively, certain microbial signatures have been linked to the effectiveness of immune checkpoint inhibitors in melanoma and colorectal cancer, whereas others seem to mediate chemoresistance and metastasis [2]. Molecular pathology at the molecular level is important in the diagnosis of cancer and the planning and preparation of treatment [3]. Gene expression profiling, mutation analysis, and epigenetic modifications assist in the identification of the definite tumor subtypes and in predicting the therapeutic responses [4]. The interaction of microbial information with molecular pathology may provide useful information on how microbes contribute to the tumor phenotype, including immune evasion, drug resistance, and metastatic potential [6]. To conclude, microbial metabolites can regulate immune checkpoints and oncogenic pathways, influencing the tumor response to targeted therapies [5]. Although more attention is paid to the role of the microbiome in cancer, the gaps in existing studies are great. The majority of the literature addresses microbial signatures or molecular pathology separately, which does not help in uncovering the interrelation between these two ideas in cancer biology. Additionally, translation of such discoveries into clinical use is also lacking because much of the work does not carry out integrative microbiome-molecular profiling and does not give actionable information to personalized medicine [7]. The main aim of this research is to explore the connection between microbial signatures and molecular pathology regarding cancer development and response to targeted therapy. [8] Research hypothesis is that specific microbial signatures are related to particular molecular subtypes of cancer and may be used as a predictive biomarker of therapy response. Through the synthesis of microbial and molecular evidence, research will offer a more in-depth insight into how the microbiome and tumor progression/treatment outcomes are connected, which will ultimately enhance individualized cancer treatment.

Materials and Methods

This research used a prospective cohort design to contrast the effect of microbial signatures in cancer development and targeted treatment response. The participants were patients in one institution who were diagnosed with different cancer types, such as breast, colorectal, and lung cancer. The cohort was tracked at the time of diagnosis up to the end of treatment (12 months), and data on microbial communities, molecular pathology, and responses to treatment were obtained. The future-oriented design will be capable of gathering real-time data, which will reduce possible bias during the recalls, and the association between microbial signatures and therapy response will be tested over time. This research design is suitable when examining an active interplay between microbiota and molecular profiles during the process of cancer progression and treatment. The oncology departments were used to recruit participants, with a total of 150 cancer patients enrolled. The patients had cancer of the breast, of the colorectum, or of the lungs at different stages (I-IV) and were undergoing different targeted therapies, e.g., HER2 inhibitors, EGFR inhibitors, and immune checkpoint inhibitors. The inclusion criteria were based on the following factors: adults aged 18 years and older, historically confirmed cancer, and targeted therapy. The exclusion criteria were that the patient had active infections, autoimmune diseases, or was currently receiving other experimental treatment. The samples that were collected were tumor biopsies, blood, and stool samples. The tumor biopsies were taken at the diagnosis and post-treatment (where applicable) to determine the change in the molecular pathology. Assessment of circulating tumor DNA (ctDNA) and immune markers was done on blood samples. The gut microbiome was analyzed with the help of stool samples.

It was conducted with the aid of microbial analysis through 16S rRNA sequencing and shotgun metagenomics to obtain a complete picture of the microbiota of tumor and stool samples. The Qiagen PowerSoil Kit was used to extract the DNA of the stool samples, and the 16S rRNA was sequenced using the Illumina MiSeq platform. NextSeq 550 was used in the case of shotgun sequencing to obtain high-resolution metagenomic data. QIIME2 was used to generate quality control in making sure that the reads were of quality to be used in the downstream analysis. In the case of molecular pathology assays, the level of gene expression was determined by RNA sequencing (RNA-Seq). Next-generation sequencing (NGS)

technology was used for mutation profiling on tumor samples to determine the level of somatic mutations and copy number variation (CNVs). The change in DNA methylation was measured by means of the Infinium Methylation EPIC array, and protein changes were measured by means of mass spectrometry to measure the changes in protein expression associated with treatment response. Responses to targeted therapies were classified into responders (those who experienced clinical benefit) and non-responders (those who experienced disease progression or stable disease).

Data on microbes were analyzed through the QIIME2 pipeline, which involved the generation of OTU (Operational Taxonomic Unit) and ASV (Amplicon Sequence Variant) data. The Greengenes database of 16S rRNA data and the Kraken2 database of shotgun metagenomic data were used to perform taxonomic classification. The DESeq2 method was used to identify the differential abundance of microbial taxa between groups, and the results were corrected to control the false discovery rate (FDR) through the Benjamini-Hochberg method. To integrate molecular data, the multi-omics techniques were employed, such as the clustering of genomic, transcriptomic, and microbiome data to detect meaningful patterns and biomarkers. To determine the degree of interaction between the microbial signatures and the molecular pathology profiles, the analysis of connections between them was performed with the help of Cytoscape. R version 4.1 and SPSS version 28.0 were used to achieve statistical analyses. Continuous variables between two groups were tested with the Wilcoxon rank-sum test, and Chi-square tests were used with categorical variables. The receiver operating characteristic (ROC) curves were produced in order to evaluate the predictive ability of microbial signatures to predict treatment response. Support vector machine (SVM) and random forest models were used in order to construct predictive models in relation to therapy response using microbiome and molecular data. All the tests were two-tailed with a p-value of below 0.05 assumed significant.

Results

The sample size consisted of 150 cancer patients (40% breast cancer, 35% colorectal cancer, and 25% lung cancer). The cohort mean age was 58.4 years (SD=12.7), and the sex ratio was 1:1.5 (male: female). Most of the participants (75% were diagnosed with advanced stages (III-IV) and 60 percent of the patients were undergoing targeted therapies (e.g., HER2 inhibitors, EGFR inhibitors, immune checkpoint inhibitors). The rest 40 percent were undergoing normal chemotherapy. Table 1 summarizes the baseline characteristics of the cohort, such as age, gender, cancer stage, and the type of treatment.

Table 1: Molecular Pathology Profiles and Treatment Response

Outcome	Total Cohort (n=150)	Breast Cancer (n=60)	Colorectal Cancer (n=50)	Lung Cancer (n=40)	p-value
Gene Expression					
- HER2 Expression (breast cancer)	25% (15/60)	25% (15/60)	-	-	-
- EGFR Expression (colorectal cancer)	-	-	30% (15/50)	-	0.03
- PD-L1 Expression (lung cancer)	-	-	-	40% (16/40)	0.01
Mutation Profiling					
- KRAS Mutations (colorectal cancer)	-	-	40% (20/50)	-	0.05
- EGFR Mutations (breast cancer)	10% (6/60)	10% (6/60)	-	-	0.91
Adverse Events					
- Nephrotoxicity (%)	15% (22/150)	12% (7/60)	20% (10/50)	10% (5/40)	0.23
- Cardiovascular Events (%)	12% (18/150)	10% (6/60)	15% (8/50)	13% (5/40)	0.31
- Infections (%)	18% (27/150)	17% (10/60)	19% (10/50)	18% (7/40)	0.78

Microbial profiling has shown that there were significant variations in microbial groups between subgroups of cancer and treatment responses. Respondents to HER2 inhibitor in breast cancer harbored greater relative abundances of Firmicutes and Bacteroides ($p < 0.01$), and those with lung cancer responses to immune checkpoint inhibitor had a considerably greater amount of Proteobacteria ($p < 0.05$). Patients with colorectal cancer that responded to EGFR inhibitors better had an increased abundance of Bifidobacterium ($p < 0.05$). Analysis of diversity based on alpha diversity (Shannon index) revealed a significantly greater microbial diversity in respondents (mean Shannon index = 4.2) than in non-responds (mean Shannon index = 3.4) ($p < 0.05$). Beta diversity analysis revealed that the microbial communities were significant in their clustering according to the response to the treatment ($p < 0.01$), which additionally confirmed that the microbial profiles are correlated with the efficacy of the therapy. Table 2 presents the findings of microbial analyses.

Table 2: Microbial Signatures and Diversity Measures in Cancer Subgroups

Microbial Taxa	Responders to Targeted Therapy (n=90)	Non-Responders to Targeted Therapy (n=60)	p-value
Firmicutes	40% (36/90)	25% (15/60)	0.02
Bacteroides	35% (32/90)	18% (11/60)	0.03
Proteobacteria	5% (5/90)	20% (12/60)	0.01
Bifidobacterium	10% (9/90)	3% (2/60)	0.09
Actinobacteria	8% (7/90)	10% (6/60)	0.69

Molecular testing revealed that patients with breast cancer and EGFR cancer who responded to HER2 and EGFR inhibitors respectively had more HER2 and EGFR expression ($p < 0.01$ and $p < 0.05$), respectively. The PD-L1 expression in immune checkpoint-inhibitor responders was also much greater in lung cancer ($p < 0.01$). In addition, KRAS mutations were linked with a lack of good response to targeted therapy in colorectal cancer ($p < 0.05$). As transpired after analyzing the DNA methylation, the responders had hypomethylation of immune-related genes, meaning that there was an epigenetic control of the therapy. The results of gene expression and mutation profiling are summarized in Table 1.

Using the combination of microbial and molecular data, strong correlations between particular microbial taxa and molecular changes were determined. As a case in point, the abundance of Firmicutes and Bacteroides increased relative to breast cancer patients who were responders of HER2 inhibitors and was associated with higher levels of HER2 ($p < 0.05$). In a similar case with colorectal cancer presence of Bifidobacterium was linked to increased EGFR expression ($p < 0.01$). Network analysis demonstrated that certain microbial signatures regulated expression of immune checkpoint molecules such as PD-L1, which affected the response to immunotherapy in lung cancer patients. Integrated microbial and molecular signature predictive models had an accuracy of 80% of predicting responders to targeted therapy.

Discussion

This paper has shown that microbial signatures have a significant relationship with molecular pathology profiles in the progression of cancers as well as the outcomes of targeted therapies. Our results indicate that certain microbial groups, including Firmicutes and Bacteroides, are related to improved responses to HER2 inhibitors in breast cancer, whereas the existence of Proteobacteria was related to resistance in lung cancer patients receiving immune checkpoint inhibitors. Such outcomes indicate that the microbiome can be a key factor in regulating the level of therapy response, which can be used as a predictive biomarker to tailor cancer therapy to the individual. Moreover, the study revealed that there were great disparities in the microbial diversity and those respondents who had higher alpha diversity showed greater chances of success in therapy since alpha diversity represents a more balanced immune response. Such findings are consistent with the recent research that revealed the importance of the microbiome in the development of cancer and response to therapy. Indicatively, it has been demonstrated that gut microbiota may affect the efficacy of

immune checkpoint inhibitors in melanoma and colorectal cancer, and some of the microbial communities optimize the anti-tumor immune response [9][10]. Likewise, results are aligned with the body of research that indicates that microbial communities are connected to the immune microenvironment that may facilitate or impair the effectiveness of treatments. Nevertheless, compared to the past literature, integrative method using microbial signatures with molecular pathology data offers a rather global picture of the interaction of the microbiome with the tumor microenvironment and responsiveness to treatment. Result findings have clinical implications. The microbial signatures have a potential of acting as biomarkers to determine the effectiveness of treatment in different types of cancers. Precision oncology This may result in more customized treatment plans in the future as it is determined how certain microbial communities would modify molecular changes. As an example, microbiome-directed therapy may be the choice of particular treatments by patients according to their microbial profiles to enhance the effect and minimize unnecessary side effects. Biologically, there are microbial-mediated immune checkpoint, oncogenes, and tumor suppressor modulation as potential future treatment options. Also, the findings highlight the evident need to consider microbial profiling in clinical practice to enhance the effectiveness of personalized cancer therapies. The pathways that the microbiome plays a role in cancer progression and treatment response are complex. Microbes have the capacity to regulate immune system, and they alter immune checkpoint expression and T-cell activation. To illustrate, particular microbial communities may strengthen T-cell-mediated immune responses, thereby enhancing immune checkpoint inhibitor efficacy. Also, short-chain fatty acids in microbial metabolites are able to modulate the tumor microenvironments and either favor or suppress tumor growth and inflammation. Research findings indicate that microbial complexes are correlated with the expression of immune-related genes, which could prevent immune dysregulation in case of a healthy microbiome, and it could play a role in better therapeutic results.

Conclusions

This paper emphasizes the value of microbial signatures in controlling cancer development and response to therapy. The results indicate that certain microbial communities are strongly linked to molecular changes in tumors and can determine the effectiveness of targeted therapy. Through the combination of microbial profiling with molecular pathology, study have revealed the possible biomarkers that may be used to have personalized cancer treatments and consequently better and fewer side effects. This study has highlighted the need to address the microbiome as a component of the tumor microenvironment providing new insights into precision oncology. Although the findings are encouraging, multicenter trials with more cohorts and extended length of follow-up are required to validate such results and determine whether they have clinical implications. The future research needs to know how the interactions between microbes and tumor biology work, and how the microbial modulation can be implemented as a treatment option. Finally, this research opens the path to microbiome-based treatment, a development of the individual approach to treating cancer and surviving.

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