Gene Expression and Pathway Analysis of Chronic Paclitaxel-induced Peripheral Neuropathy in Breast Cancer Survivors

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Background and Purpose

The prevalence of chemotherapy-induced peripheral neuropathy (CIPN) in cancer survivors is as high as 60%. CIPN is the most debilitating symptom reported oncology patients and can lead to treatment discontinuation. No interventions are available to treat CIPN. A critical barrier to the development of efficacious interventions is the lack of understanding of the mechanisms underlying CIPN. In this pilot study, we evaluated the molecular characteristics associated with paclitaxel-induced peripheral neuropathy (PIPN).

The objective of this pilot study was to compare differential gene expression (GE) between breast cancer (BC) survivors with and without PIPN with a focus on mitochondrial dysfunction.

Methods

Of the 1450 cancer survivors who were screened, 754 were enrolled, and 623 completed the self-report questionnaires and the study visit in the Parent grant (R01 CA151692). The National Coalition for Cancer Survivorship's definition of cancer survivor was used in this study (i.e., a person is a cancer survivor from the moment of diagnosis through the balance of life). Data from a randomly selected sample of breast cancer survivors with (n=25) and without (n=25) chronic PIPN were used in this analysis.

Demographic and Clinical Designs

Survivors with PIPN had changes in sensation and/or pain in their feet and/or hands of >3 months duration following the completion of CTX; had a rating of >3 on a 0 to 10 numeric rating scale (NRS) for any one of the following sensations from the Pain Quality Assessment Scale (PQAS;81 i.e., numb, tender, shooting, sensitive, electrical, tingling radiating, throbbing, cramping, itchy, unpleasant); if they had pain associated with the CIPN, had an average pain intensity score in their feet and/or hands of >3 on a 0 to 10 NRS.

Survivors provided information on demographic characteristics and completed the Alcohol Use Disorders Identification Test, the Karnofsky Performance Status (KPS) scale, and the Self-Administered Comorbidity Questionnaire (SCQ).

Gene Expression Measurement

Total RNA was isolated from peripheral blood samples collected at the time of the assessment using PAXgene® Blood RNA tubes and treated to deplete cytoplasmic ribosomal RNA and human globin mRNA. The 50 samples were multiplexed into a single pool and sequenced using the Illumina HiSeq 4000 (100bp single end).

Reads were aligned to the GRCh38 assembly of the human genome and Gencode v24 primary assembly of the transcriptome using the STAR aligner. RNA abundance was estimated as read counts summarized at the gene level using featureCounts and normalized (TMM). Technical artifacts were estimated using SVA.

Differential gene expression was assessed with edgeR. Significant demographic and clinical characteristics and SV were included in the model. Differentially perturbed KEGG signaling pathways were evaluated using a cutoff-free pathway impact analysis with Pathway Express.

Results

Differences in Demographic and Clinical Characteristics

- BC survivors with PIPN were significantly older; more likely to be unemployed; reported lower alcohol use; had a higher BMI, and poorer functional status; and had a higher number of lower extremity sites with loss of light touch, cold, and pain sensations, and higher vibration thresholds.
- No between group differences were found in the total dose of paclitaxel received or in the percentage

Results (Continued)

Differences in Gene Expression

- We identified 198 (5% FDR) and 140 (1% FDR) DE genes
- We identified 153 (5% FDR) and 21 (1% FDR) DE pathways

Table 1. Differentially Expressed Mitochondrial Dysfunction Related Genes Between Breast Cancer Survivors With and Without PIPN.

Symbol	Name	Entrez ID	Log FC	P-value (1% FDR)
PRDX5	Peroxioredoxn 5	25824	-0.987	0.0098
GLRX5	Glutaredoxin 5	51218	-1.703	0.0062
FIS1	Fission, mitochondrial 1	51024	-1.062	0.0062
UBB	Ubiquitin B	7314	-1.374	0.0061

Figure 1. Pathway level statistics for Mitophagy - Animal pathway (KEGG hsa04137). The color of each node represents the perturbation (red = positive, blue = negative) and the shade represents the strength of the perturbation.

Table 2. The five most Differentially Perturbed Mitochondrial Dysfunction Related KEGG Pathways Between Breast Cancer Survivors With and Without PIPN .

Symbol	Name	Tot. Perturbation	pPert (1% FDR)
hsa04137	Mitophagy – animal	11.78	< 0.0004
hsa04150	mTOR signaling pathway	9.60	< 0.0004
hsa04152	AMPK signaling pathway	8.99	< 0.0004
hsa04151	PI3K-AKT signaling pathway	8.04	< 0.0004
hsa04066	HIF-1 signaling pathway	7.45	< 0.0004

Discussion

This study is the first to provide molecular evidence that a number of mitochondrial dysfunction mechanisms identified in preclinical models of various types of neuropathic pain including CIPN, are found in breast cancer survivors with persistent PIPN.

Our findings support the preclinical mechanisms associated with PIPN and suggest genes for validation and as potential therapeutic targets

Conclusions

Chronic PIPN is associated with GE changes in peripheral blood. Our findings support previously hypothesized mechanisms of PIPN and suggest additional genes for validation and potential therapeutic targets.

Acknowledgements

Research reported in this study was supported by grants from the National Cancer Institute (Miaskowski, R01CA134900, K05CA168960) and American Cancer Society (Kober, IRG-97-150-13). Dr. Miaskowski is an American Cancer Society Clinical Research Professor.