



HHS Public Access

Author manuscript

J Occup Environ Med. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

J Occup Environ Med. 2016 April ; 58(4): 344–350. doi:10.1097/JOM.0000000000000705.

Transcriptional Pathways Altered in Response to Vibration in a Model of Hand-Arm Vibration Syndrome

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Abstract

Objective—The aim of this study was to use an established model of vibration-induced injury to assess frequency-dependent changes in transcript expression in skin, artery, and nerve tissues.

Methods—Transcript expression in tissues from control and vibration-exposed rats (4 h/day for 10 days at 62.5, 125, or 250 Hz; 49 m/s², rms) was measured. Transcripts affected by vibration were used in bioinformatics analyses to identify molecular- and disease-related pathways associated with exposure to vibration.

Results—Analyses revealed that cancer-related pathways showed frequency-dependent changes in activation or inhibition. Most notably, the breast-related cancer-1 pathway was affected. Other pathways associated with breast cancer type 1 susceptibility protein related signaling, or associated with cancer and cell cycle/cell survivability were also affected.

Conclusion—Occupational exposure to vibration may result in DNA damage and alterations in cell signaling pathways that have significant effects on cellular division.

Occupational exposure to repeated-bouts of hand-transmitted vibration (HTV) results in a number of different disorders, including cold-induced vasospasms in the fingers and hands, and peripheral neuropathy. These symptoms have been referred to as hand-arm vibration syndrome (HAVS 1). However, exposure to HTV can also result in an increased sensitivity to environmental stimuli, such as cold and excessive noise, which in turn can enhance the effects of vibration and result in hypersensitivity of the sympathetic nervous system.² Depending on the frequency, amplitude, and duration of the exposure, occupational exposure to vibration can also induce degeneration of bone³ and it is associated with an increased risk of arthritis and muscle wasting.^{1,4,5}

Epidemiological studies have shown that there is an increased risk of developing the most prevalent symptoms of HAVS (vasospasms and sensorineural changes) when the primary vibration frequency emitted by tools is in the range of 60 to 400 Hz.^{6–8} On the basis of the results of experiments performed in humans and mathematical modeling studies, it has been

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There are no conflicts of interest, or additional funding sources to declare.

suggested that the increased risk of injury that occurs with exposure to mid-range vibration frequencies may be because the resonant frequency of the fingers and hands are in this range.^{9–12} Shear and bending stress and strain on vibration-exposed tissues is greatest at the resonant frequency, and the increase in this tissue stress has been proposed to lead to an increase in tissue injury or dysfunction.^{12–14} Results from studies using a well characterized animal model of vibration-induced dysfunction also support this hypothesis; Studies using a rat-tail vibration model have demonstrated that 10 days of exposure to vibration at 62.5, 125, or 250 Hz resulted in frequency-dependent changes in peripheral vascular and sensorineural tissues, with changes in vascular and sensorineural function being most prevalent at or near the resonant frequency (ie, 250 Hz).^{15–17}

Changes in gene expression also tend to be frequency-dependent.¹⁶ Total rat genome arrays have been used to assess frequency-dependent changes in gene transcription in tail skin, peripheral nerves, and arteries in these studies. These data were analyzed and used to identify specific pathways that are associated with vibration-induced vascular and peripheral nerve injury. However, rapid advancements in the ability to collect and measure transcript levels using gene arrays and identify the molecular pathways these genes play a role in, has allowed researchers to predict how various factors affecting cellular and intracellular functions may contribute to the development of diseases, or predict toxicity and/or pharmaceutical potential.¹⁸ Because of advances in our knowledge, we reanalyzed the array data collected from the nerves and arteries (initial data presented in)^{15,18} to identify new or additional cellular pathways that may be affected by vibration exposure and identify potential diseases that may arise from alterations in these pathways. In addition, new array data collected from the skin of rats that had been exposed to vibration at 62.5, 125, or 250 Hz were analyzed to determine what major cellular pathways were activated in response to vibration at various frequencies, and the downstream disease processes that also might be affected by these changes in gene expression. We chose to use those frequencies because previous data from our laboratory demonstrated that the physical responses of the tail tissues to vibration transmission at these three frequencies were different (ie, the degree of shear and bending stress was different at each frequency).

METHODS

Animals

Male Sprague-Dawley [H1a:(SD) CVF rats; 6 weeks of age at arrival; Hilltop Lab Animals, Inc, Scottsdale, PA) were used in this study. Rats were maintained in a colony room with a 12 : 12 reversed light:dark cycle (lights off 0700 h) with Teklad 2918 rodent diet and tap water available ad libitum, at the National Institute for Occupational Safety and Health (NIOSH) facility, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Rats were allowed to acclimate to the laboratory for one week before beginning the study. All procedures were approved by the NIOSH Animal Care and Use Committee and were in compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the NIH Guide for the Care and Use of Laboratory Animals.

Vibration Exposures

The equipment and protocol for exposing animals to vibration has been previously described.^{17,19} Briefly, rats were placed in Broome style restrainers for 1 to 2 hours a day for 5 days to acclimate them to the restrainer. After a week of acclimation to restraint, rats were randomly assigned to a cage-control group, restraint-control group, or one of three vibration groups, where vibration exposure frequencies were 62.5, 125, or 250 Hz ($n = 4$ rats/group). Power calculations from previous studies demonstrated that significant changes in transcription could be detected using four animals per group, and these changes in transcription were associated with changes in morphology and/or function.^{16,20} During exposures, vibrated rats had their tails secured to a vibrating platform. Restraint-control rats were placed in the chambers along with vibrated rats, but their tails were strapped to platforms that were mounted onto nonvibrating isolation blocks, and cage-control rats were maintained in the colony room. Each exposure was performed between 0900 and 1300 hours, and was 4 hours in length. The acceleration used for all vibration frequencies was 49 m/sec^2 root mean squared or r.m.s. (unweighted acceleration; sinusoidal vibration in the vertical direction). After each exposure, rats were returned to their home cages and housed in the colony room. Rats were exposed to vibration or restraint for 10 consecutive days. We also chose to test the effects of these frequencies because we have demonstrated that vibration transmissibility was different at these frequencies and thus we can use these exposures to determine how frequency-dependent changes in stress and strain affect injury.¹⁷

Tissue Samples

One hour following the final exposure, rats were deeply anesthetized using pentobarbital (100 mg/kg) and exsanguinated by cardiac puncture. Ventral tail arteries were dissected from the C9 to C10 region. This segment was chosen because the biodynamic response (ie, the displacement of the vibrated appendage/the displacement of the vibrating platform) to vibration is frequency dependent in this region of the tail, and shows different responses to the three exposure frequencies.¹⁷ The skin, ventral tail artery, and ventral tail nerve were immediately dissected from the tail, placed into cryovials, frozen in liquid nitrogen, and stored at -80°C until RNA was isolated.

Illumina Rat Expression Arrays

RNA was isolated and purified using the RNeasy Fibrous Tissue Mini Kit (Qiagen Sciences, Valencia, CA). RNA concentrations were determined using a ND-1000 UV/Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE). Purity and integrity of the RNA were measured using the Agilent 2100 bio-analyzer and an RNA LabChip Kit (Agilent Technologies, Santa Clara, CA). All samples had a RNA Integrity Number (RIN) of 9.0 or higher. Samples containing 375 ng total RNA were amplified and labeled using the TotalPrep RNA amplification kit (Ambion, Austin, TX) following the standard kit protocols. The hybridization mix containing 750 ng of labeled amplified RNA was prepared and loaded onto the Sentrix RatRef-12 Expression Beadchip (Illumina, San Diego, CA) using the supplied reagents, hybridized for 20 hours at 58°C in an Illumina Hybridization Oven, and washed and dried according to the Whole-Genome Gene Expression with IntelliHyb Seal System Manual (Illumina). Chips were taken to Allegheny Singer Research Institute

(Pittsburgh, PA) and scanned using an Illumina Beadstation-500G BeadArray reader (Illumina).

Files containing fluorescent intensity from the bead scanner were loaded into Beadstudio (Framework version 3.0.19.0 Gene Expression module v.3.0.1.4). Housekeeping, hybridization control, stringency, and negative control genes were checked for proper quality control. Both technical controls and sample transcripts are located on multiple beads. There are 15 to 30 replicates for each control or transcript and an average is calculated from these replicates and used for analyses. Bead array expression data were exported with mean fluorescent intensity across like beads, and bead variance estimates into flat files for subsequent analyses.

Illumina BeadArray expression data were analyzed in Bio-conductor using the “lumi” and “limma” packages. The “lumi” Bioconductor package was specifically developed to process Illumina microarrays and covers data input, quality control, variance stabilization, normalization, and gene annotation. The background correction method was “force positive” and was used to force all expression values to be positive by adding an offset (minus minimum values plus 1). Data were then transformed using a variance-stabilizing transformation (VST). The VST method takes advantage of the technical replicates available on an Illumina microarray.²¹ Data normalization then proceeded using the robust spline normalization algorithm, which combines the features of quantile and loess normalization.²¹ Before subsequent analyses including differential expression analysis, unexpressed genes are filtered out. Normalized data were then analyzed using the “limma” package in Bioconductor.²² Limma was used to fit a linear model for each gene, generate group means of expression values, which were a *P* value less than 0.05 and a fold change of ± 1.25 between the groups. These fold changes were chosen to include genes that were previously identified as being associated with vibration-induced changes in vascular or sensori-neural physiology. These lists of genes and their associated statistics were utilized as input for subsequent bioinformatic analyses using Ingenuity Pathways Analysis (Ingenuity Systems, www.ingenuity.com).

RESULTS

Data for individual transcripts in the skin that were significantly affected by vibration are provided in Table 1 (ie, transcripts that displayed changes in response to vibration, 1.4 fold or greater change than restraint control animals and $P < 0.01$). Heat maps were generated for these transcripts using the fold change in individual transcripts as compared with cage controls. Reductions in the fold change in transcripts are represented in blue; the greater the reduction in transcript number, the darker the color. Greater increases in fold changes in transcript number are represented by red; the brighter color indicates a greater fold increase (as compared with cage controls). The heat maps in Fig. 1 are displayed on the basis of their potential involvement in different cancers (Fig. 1A to D). These heat maps demonstrate how genes altered by vibration have been associated with cancer in a number of different tissue types. To identify the role of various genes in different cancers, internet sites showing the prevalence of immunohistochemical staining in certain cancers, and references from the literature were used.^{23–29}

When the bioinformatics data from all tissues were examined, there were some common pathways that were identified. In the skin (Fig. 2) and artery (Fig. 3), the primary pathway affected was the breast cancer type 1 susceptibility protein (BRCA1) in the DNA damage response pathway, while exposure to vibration resulted in activation, particularly at 62.5 and 250 Hz. The opposite findings were seen in the artery (Fig. 3); restraint resulted in the inhibition of genes involved in the BRCA1 pathway, whereas whole vibration resulted in an increase.

Table 2 summarizes the diseases and other cellular functions that are altered by restraint or vibration exposure in the skin, artery, and nerve. The top pathway that displayed a significant *P* value was identified for each tissue, and is listed in the table (values highlighted in yellow). The four to five top canonical pathways related to disease and biological functions for each tissue type are presented in Fig. 4. Pathways with *Z*-scores less than -1.25 or more than 1.25 were considered to be potentially significant and were included in the results. In all tissues, many of the transcripts expressed were in pathways involved in regulating cell replication and/or cell survival and death.

The ataxia telangiectasia mutated (ATM) pathway was also affected by vibration in the skin and artery of rats. In the skin, restraint resulted in an increase in the activation of genes within this pathway, while vibration at 250 Hz resulted in an inhibition of this pathway. In the artery, exposure to vibration at 62.5 Hz resulted in an activation of the ATM pathway. Restraint and vibration at 125 and 250 Hz did not significantly affect activation of this pathway in the artery. The cyclin-dependent kinase 5 (CDK5) pathway was also affected by vibration at 65 Hz.

Vibration-induced changes in specific transcripts for the artery and nerve previously were published.¹⁶ Reanalyses of the data from these tissues did not identify any new transcripts that were affected by vibration. However, there were differences in the pathways that were activated. Pathways regulating general metabolism, oxidative stress, and vascular function were still important.^{16,30} However, reanalyses of the data using Ingenuity 2015 showed that vibration also affected pathways involved in cancer, apoptosis, and cell cycle regulation. Other cancer-related pathways activated in the artery included the nuclear factor kappa light-chain enhancer of activated B cells (NF κ B) signaling pathway, which was activated with exposure to vibration at 125 and 250 Hz that resulted in significant activation of this pathway. Exposure to vibration at 125 Hz resulted in an increase in genes that are involved in cell cycle: G1/S checkpoint regulation. In the nerve (Fig. 4), pathways that were affected by vibration and that may affect the risk of developing cancer included organismal death and cell viability pathways.

Figure 5 is a hypothetical model showing how vibration-induced pathways in various genes and their associated pathways may interact to alter the risk of developing cancer.

DISCUSSION

The goal of this study was to use updated information from the Ingenuity gene array database to determine how transcript expression that was altered by vibration and/or restraint

fit into pathways that are associated with disease or cellular function/dysfunction. Analyses of these data showed that many of the pathways affected (either activated or inhibited) were pathways involved in regulating cell division or in cell death, and that these effects were frequency dependent.

A hypothetical drawing of how the cellular pathways that were changed in both the skin and the artery may interact can be found in Fig. 5. Studies have demonstrated that carcinogens, such as ultraviolet (UV) radiation, can result in DNA breakage. In response to the DNA breakage that occurs because of an insult, CDK5 can be activated and phosphorylate ATM. In turn, ATM can activate NF κ B to regulate apoptosis. ATM can also activate the BCRA1 and BRCA2 pathways, which in turn may act as transcription-regulating factors that affect DNA repair and cell cycle.³¹ Because vibration seems to affect similar pathways, it is possible that vibration may act in a manner similar to UV radiation, and alter pathways associated with regulation of cell cycle, DNA repair, and cancer. There is evidence that exposure to whole-body vibration (WBV) may increase the risk of developing prostate cancer and cancers of the abdomen.³² Vibration also stimulates vascular and bone growth and remodeling.^{1,20} Thus, it is not surprising that pathways involved in cell cycle regulation and DNA repair were altered by vibration exposure.

One way vibration may affect the activation or inhibition of the ATM and BRCA1 pathways is through the *Per1* gene. The *Per1* gene also interacts with the ATM pathway, which includes a number of tumor suppressor genes.³³ *Per1* was identified as a transcription factor involved in the maintenance of circadian rhythms. However, a number of studies have demonstrated that the disruption in circadian rhythms, and in cellular mechanisms involved in regulating circadian clock function, is associated with an increased risk of a number of different cancers in employees performing shift work.^{26,33} Alterations in the expression of, or mutations in genes involved in regulating circadian clock function, are associated with changes in intracellular inflammation, apoptosis, and aberrations in cell proliferation, metabolism, and drug resistance in human cancer cells.^{24–28,33}

Although the BRCA1 pathway is most recognized for its role in affecting the risk of developing breast and ovarian cancer, the *Brcal* gene is located in a number of different tissues. The tissue-specific locations that have been examined in mice, humans, and cynomolgus monkeys are slightly different, but the distribution of this gene is not limited to breast and ovarian tissues in any of these species. *Brcal* in primates is expressed in virtually every physiological system and may affect the risk of developing a number of different cancers.³⁴ For example, in adults, the BRCA1/2 pathways have been shown to play a role in the development of prostate and skin cancer^{29,35}; Activation of the BRCA1 pathway appears to protect against the development of cancer, and inhibition of this pathway accelerates the rate of aberrant cell growth.³¹ The BRCA1/2 pathways are not only involved in regulating cancer development, but they also play a role in other biological pathways. For example, early during the developmental process, the BRCA1 pathway is involved in rapid cell division during specific stages of embryogenesis in the mouse, and BRCA1 deletions result in death of the embryo.

Many of the individual transcripts that changed also play a role in regulating cell cycle or play a role in pathways that affect tumorigenesis. For example, *Sfn* has been shown to regulate cell cycle arrest. Decreases in this transcript and protein have been linked to the development of pancreatic,²³ skin, and lung cancers.³⁶ *Rasd1* is involved in mediating dexamethasone-induced changes in cell morphology and increases in this gene have been associated with an increase in apoptosis in prostate and breast cancer cells.^{37,38} *Pmel* is involved in maintaining the fiber matrix of melanocytes, and changes in this protein have been linked to the regulation of cellular pathways mediating the development of melanoma.^{39,40} Reductions in the *Per1* gene have been associated with dysregulation of cell cycle and an increased risk of developing breast and gastric cancers.^{24,27,33} Changes in *F2r* are not involved in cell cycle or associated with cancer, but this gene is involved in regulating thrombin production and thus may play a role in the atherosclerotic pathway. Another transcript that was altered, *Thhc1*, is involved in regulating hair thickness. Unlike the human finger, the rat tail has large guard hairs. These hairs are associated with sensory receptors in the rat tail, and previous work has demonstrated that exposure to impact vibration affects innervation of the hair follicle.^{16,41} Thus, changes in the expression of this transcript could result in alterations in sensory perception in the rat, or in nonglabrous skin in humans.

The question is why does vibration activate or inhibit pathways that are involved in regulation of cell cycle and cancer? The mechanical energy absorbed by tissues when exposed to vibration, particularly vibration at the resonant frequency, has been shown to induce vascular smooth muscle and peripheral nerve remodeling.^{20,30} Because these processes involve growth and cell division, activation of cellular pathways that play a part in repair or remodeling mechanisms are activated in response to vibration-induced injury and dysfunction. These same pathways might also stimulate changes in cell division in other tissues. We have also shown that exposure to vibration results in an increase in the generation of reactive oxygen species (ROS) in the artery and the nerve.^{15,16} A number of studies have linked increases in ROS to activation of pathways involved in tumorigenesis.^{42,43} Thus, vibration may also alter cycle/tumor-related pathways by inducing oxidative stress.

Although HTV has not been associated with the development of cancer, there are studies suggesting that exposure to WBV, similar to that experienced by truck drivers, and operators of heavy construction, farm, and mining equipment, may be associated with an increased risk of developing colon cancer, prostate cancer, and cancer in other abdominal organs.^{32,44} However, there are conflicting studies that suggest there may be no connection between vibration and the risk of cancer.⁴⁵ The findings of our study in rats suggest that depending on the specific frequency of exposure, pathways regulating aberrant cell growth and the development of cancer may either be activated or inhibited. On the basis of the current epidemiological and experimental data, it is difficult to determine whether these pathways are activated or inhibited to stimulate repair and regenerative processes or whether they are acting to induce aberrant cell growth that eventually results in tumor formation. It is also possible that initially, vibration stimulates repair and regenerative processes, but that with continued exposures, regenerative processes become unregulated and result in aberrant growth of cells and physiological dysregulation. Additional work determining how vibration

energy is transmitted through the body, and studies done to determine the resonant frequencies of various tissues may provide information that can be used to reduce the risk of developing cancer, or be used in the development of new cancer treatments.

Acknowledgments

This work was internally funded by the National Institute for Occupational Safety and Health (NIOSH). The content of this publication does not necessarily reflect the views or policies of the NIOSH.

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Transcript	Restraint	62.5 Hz	125 Hz	250 Hz
Krtap6-2	Dark Blue	Light Blue	Red	Dark Red
Krtap7-1	Dark Blue	Light Blue	Red	Dark Red
Ttn	Light Blue	Light Red	Light Blue	Light Red
EFHD1	Dark Blue	Light Red	Red	Light Red
engase	Light Red	Light Red	Light Red	Light Blue
adipoq	Light Red	Light Red	Light Red	Red
Reep5	Light Red	Light Blue	Light Red	Light Blue
cnih1	Light Red	Light Blue	Light Blue	Light Blue
Pmel	Light Red	Light Blue	Light Blue	Dark Blue
Per1	Light Red	Light Blue	Light Blue	Dark Blue

A Skin cancers

Transcript	Restraint	62.5 Hz	125 Hz	250 Hz
Krtap6-2	Dark Blue	Light Blue	Red	Dark Red
Krtap7-1	Dark Blue	Light Blue	Red	Dark Red
EFHD1	Dark Blue	Light Red	Red	Light Red
Pygm	Dark Blue	Light Red	Red	Light Red
adipoq	Light Red	Light Red	Light Red	Red
Reep5	Light Red	Light Blue	Light Red	Light Blue
cnih1	Light Red	Light Blue	Light Blue	Light Blue
Rasd1	Light Red	Light Blue	Light Blue	Dark Blue
Per1	Light Red	Light Blue	Light Blue	Dark Blue

B Breast cancer

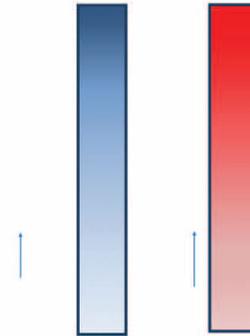
Transcript	Restraint	62.5 Hz	125 Hz	250 Hz
adipoq	Light Red	Light Red	Light Red	Red
Tchh	Light Red	Light Red	Light Red	Light Red
Reep5	Light Red	Light Blue	Light Red	Light Blue
cnih1	Light Red	Light Blue	Light Blue	Light Blue
Mrpl18	Light Red	Light Blue	Light Blue	Light Blue
Per1	Light Red	Light Blue	Light Blue	Dark Blue

C Cancers of abdominal organs

Transcript	Restraint	62.5 Hz	125 Hz	250 Hz
adipoq	Light Red	Light Red	Light Red	Red
Tchh	Light Red	Light Red	Light Red	Light Red
Sfn	Light Red	Light Red	Light Red	Light Red
EFHD1	Dark Blue	Light Red	Red	Light Red
Fr2	Light Red	Light Blue	Light Blue	Red
Reep5	Light Red	Light Blue	Light Red	Light Blue
cnih1	Light Red	Light Blue	Light Blue	Light Blue
Mrpl18	Light Red	Light Blue	Light Blue	Light Blue
Per1	Light Red	Light Blue	Light Blue	Dark Blue
Rasd1	Light Red	Light Blue	Light Blue	Dark Blue

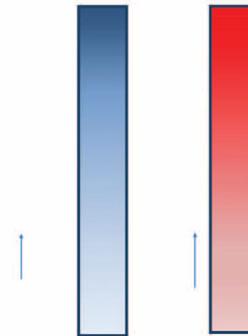
D Reproductive tissues (prostate, cervical, uterine)

Reduction in transcript number



Increase in transcript number

Reduction in transcript number



Increase in transcript number

FIGURE 1.

Heat maps of transcripts that changed in the skin in response to restraint or vibration exposure of different frequencies are displayed in each figure (changes are from cage control condition). Transcript names that are in color are transcripts that appear to be associated with changes in cell cycle regulation or cancer development in more than one region of the body. Transcript names that are in black may only be associated with cell cycle regulation or cancer in a single region.

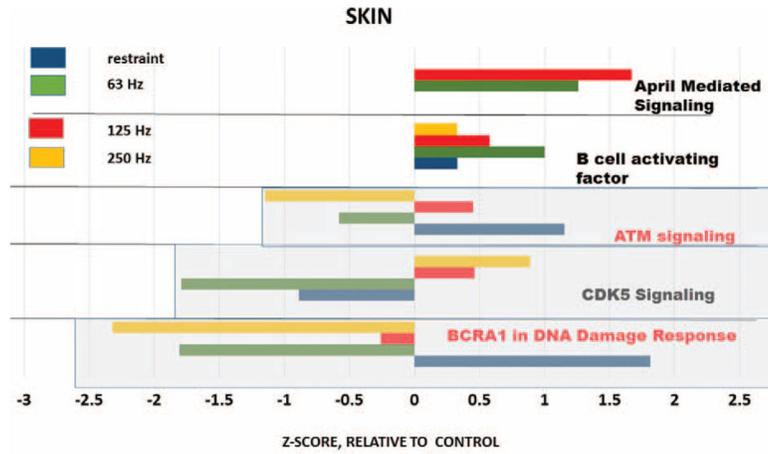


FIGURE 2. Changes in transcriptional pathways relative to unexposed controls in the skin are presented in this graph. The five pathways shown had the greatest number of transcripts changing in response to restraint or vibration. Pathways highlighted in gray may play a role in mediating cancer risk. Missing bars mean that there were no significant changes in these pathways at those frequencies.

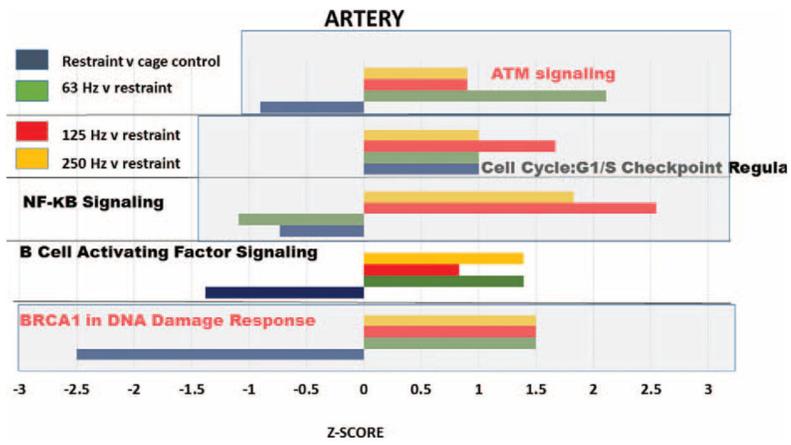


FIGURE 3. Changes in transcriptional pathways relative to unexposed controls in the ventral tail artery are presented in this graph. Pathways highlighted in gray may play a role in mediating cancer risk.

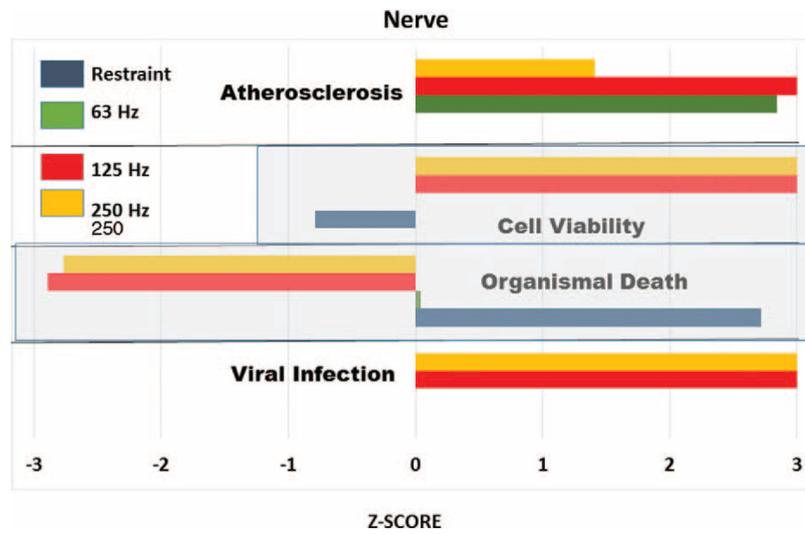


FIGURE 4. Changes in transcriptional pathways relative to unexposed controls in ventral tail nerve. The pathways highlighted in gray play a role in cell survival/replication and cell death. The transcripts in these pathways may be involved in development or mediate the risk of developing cancer by affecting cell cycle. Missing bars mean that there were no significant changes in these pathways at those frequencies.

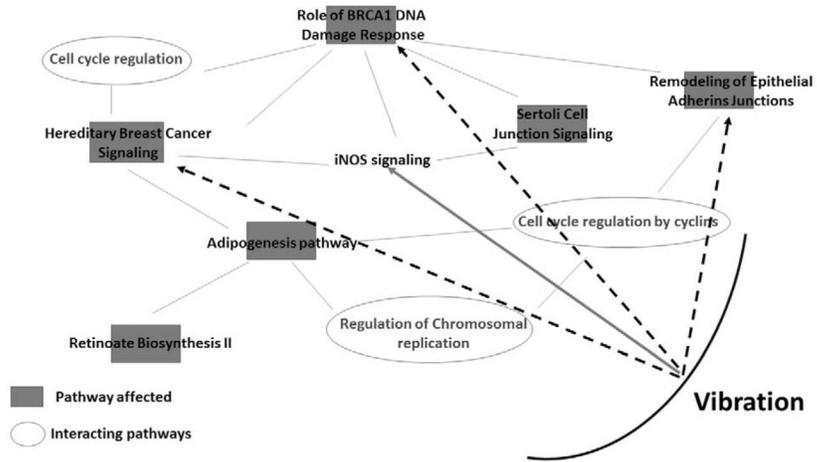


FIGURE 5. Various pathways that are affected by restraint and vibration and may interact to regulate development, cell division, and the risk of developing cancer are presented in this figure. The blue lines between pathways represent known interaction, the dashed lines represented hypothesized interactions, and the red line represents one mechanism by which vibration may act to affect these various pathways.

TABLE 1

The Number of Transcripts or Molecules Affected in Each Tissue Based on Classification by Disease Pathway

Classification	Pathway Function	Number of Molecules Identified		
		Skin	Artery	Nerve
Disease and disorders	Cancer	392	1231	853
Disease and disorders	Gastrointestinal disease			611
Molecular and cellular	Gene expression	423	440	
Molecular and cellular	Cell growth and proliferation			353
Molecular and cellular	Embryonic development	245	279	

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TABLE 2

Transcripts in the Skin that were Affected by Vibration

Transcript	63 Hz	125 Hz	250 Hz
Adiponectin (<i>Adipoq</i>)			-1.49
Cornichon family AMPA receptor auxiliary protein (<i>Cnih1</i>)	-1.50		
E-F hand domain containing protein (<i>efhd7</i>)		+1.68	
Keratin associated protein 6-2 (<i>Krtap6-2</i>)		+2.58	+2.64
Keratin associated protein 7-1 (<i>Krtap7-1</i>)		+3.2	+2.0
Protease activated receptor 2 (<i>F2r</i>)		-1.47	
Mitochondrial ribosomal protein 18 (<i>Mrp118</i>)	+2.079	+1.37	
Myosin heavy chain 11 smooth muscle (<i>Myh11</i>)	+1.42		+1.42
Myotilin (<i>Myot</i>)	+1.46		+1.46
Period 1 (<i>Per1</i>)	-1.27	-1.56	-1.55
Pleckstrin (<i>Plekht2</i>)		-1.43	
Premelanosome (<i>Pmel</i>)		-1.46	-2.01
Phosphorylase glycogen muscle (<i>Pygm</i>)	+1.73		+1.73
Dexamethasone-induced Ras-related protein (<i>Rasd1</i>)		-1.50	
Retinol binding protein 2 (RBP2)		+1.45	
Stratifin (SFN)	-1.48	+1.40	
Sodium-coupled monocarboxylate transporter 1 (SLC5A8)		+1.51	
Titan (TTN)	+2.03		+2.03

The values in the columns represent the fold change in expression. Values with a fold change greater than ± 1.4 and $P < 0.01$ (in bold type) were considered significant. Transcripts that showed changes in response to vibration in the ventral tail artery and nerve can be found in Krajnak et al.¹⁶