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MICROARRAY CLUSTER ANALYSIS OF IRRADIATED GROWTH PLATE ZONES FOLLOWING LASER MICRODISSECTION

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Abstract

Purpose—Genes and pathways involved in early growth plate chondrocyte recovery after fractionated irradiation were sought as potential targets for selective radiorecovery modulation.

Methods and Materials—Three groups of six 5 week male SD rats underwent fractionated irradiation to the right tibiae over 5 days totaling 17.5 Gy and then were killed at 7, 11 and 16 days following the first radiotherapy fraction. The growth plates were collected from the proximal tibiae bilaterally and subsequently underwent laser microdissection to separate reserve, perichondral, proliferative, and hypertrophic zones. Differential gene expression was analyzed between irradiated right and non-irradiated left tibia using RAE230 2.0 GeneChip microarray, compared between zones and time points and subjected to functional pathway cluster analysis with real-time PCR to confirm selected results.

Results—Each zone had a number of pathways showing enrichment following the pattern of hypothesized importance to growth plate recovery, yet few met the strictest criteria. The proliferative and hypertrophic zones showed both the greatest number of genes with a 10 fold right/left change at 7 days after initiation of irradiation and enrichment of the most functional pathways involved in bone, cartilage, matrix and/or skeletal development. Six genes confirmed by real-time PCR to have early upregulation included Igf2, Col1a2, Mmp9, Pthr1, Fmod, and Agc1.

Conclusions—Nine overlapping pathways in the proliferative and hypertrophic zones (skeletal development, ossification, bone remodeling, cartilage development, extracellular matrix structural constituent, proteinaceous extracellular matrix, collagen, extracellular matrix, and extracellular matrix part) may play key roles in early growth plate radiorecovery.

Keywords

Growth plate; microarray; chondrocytes; rat; irradiation

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Conflicts of Interest Notification: None

Introduction

Longitudinal bone growth results from orderly coordination of chondrocyte proliferation and hypertrophy, calcification of the matrix, vascular invasion, and completion of endochondral bone formation in the growth plate. Despite recent advances, a complete understanding of the factors regulating progression in the growth plate has not been achieved. Even less is understood about the mechanisms of recovery of the growth plate from radiation damage. What is known is that children who receive radiotherapy for bone or soft-tissue sarcomas in areas close to a growth plate are at high risk for developing growth arrest, angular deformity, and/or limb length discrepancy. (1,2,3)

Some capacity for growth plate recovery after irradiation appears to exist, based both on the variability of clinical outcomes following irradiation of the growth plate and on evidence from our own work in the weanling Sprague-Dawley rat model. Our recent work has focused on obtaining a better understanding of early growth plate recovery in order to develop potential selective radiorecovery agents for clinical use during radiotherapy treatment of pediatric solid tumors. It is postulated that identification of key early upregulated genes may provide an understanding of the mechanism of this recovery and lend itself to the development of novel radiorecovery agents.

In our earlier work a single fraction model was utilized for simplicity's sake. (4,5) Examination of gene recovery isolated to the proliferative (PZ) and hypertrophic (HZ) zones suggested a specific pattern of radiorecovery involving phased upregulation of both matrix elements and growth factors/cytokines. Since fractionation is the clinical norm; we sought in this manuscript to report the more clinically relevant pattern of radiorecovery following fractionated irradiation. In addition, since key growth plate regulatory elements are thought to be generated in the perichondrium and reserve zone, these zones were also isolated by laser microdissection in the current report.

The purpose of this report was to utilize laser microdissection and microarray to separate and characterize gene expression in four zones of the growth plate after fractionated irradiation, as well as to determine enrichment of gene ontology functional groups in significantly changed genes. Our hypothesis was that differential upregulation of specific gene expression exists between irradiated and nonirradiated reserve zone (RZ), proliferative zone (PZ), perichondrium (PC) and hypertrophic zone (HZ) chondrocytes and that some factors potentially vital to growth plate recovery would follow a pattern of early upregulation followed by a decrease in expression.

Methods

All animal procedures were reviewed and approved by the Institutional Use and Care of Animals Committee (IUCAC). Three groups of six 5 week male SD rats underwent fractionated irradiation to the right tibiae over 5 days totaling 17.5 Gy. The left side tibiae served as non-irradiated controls. At 7, 11 and 16 days following the first radiotherapy fraction, the animals were killed by carbon-dioxide asphyxiation, and the growth plates were collected from the proximal tibiae bilaterally and immediately frozen in liquid nitrogen and stored at -70°C. The entire collecting process was completed within two minutes.

These timepoints were chosen based upon previous work showing that, from a histologic perspective, the growth plate reaches its most disorganized, paucicellular state at approximately one week after irradiation. Gradual recovery has been noted thereafter, with clones of regenerating chondrocytes clearly identified beginning at 2 weeks after irradiation. Hence, in order to find genes that might play a role in stimulating growth plate recovery, we hypothesized

that the key genes would be upregulated by 7 days but would gradually return toward normal by 11 and 16 days after irradiation.

Laser Microdissection

Serial sections (6 μm for laser microdissection) were cut on a Leica CM3050 cryostat immediately before the planned laser microdissection. The laser microdissection (LMD), RNA extraction and Gene Chip Hybridization procedures were previously described.(6) For each of the four zones at each of the three time points, equal amounts of RNA from the three samples derived from three different limbs on each side (right and left) were pooled to create a single sample to reduce the individual variability and sampling errors, resulting in eight samples of RNA from the six limbs at each time point, 24 samples in all.(Figure I) The twenty-four RNA samples included the right RZ at 7 days, left RZ at 7 days, right PZ at 7 days, left PZ at 7 days, right HZ at 7 days, left HZ at 7 days, right PC at 7 days, and left PC at 7 days, and identical sets for 11 and 16 days. Each pooled sample had 30-50 ng RNA.

Real-Time RT-PCR

Real time quantitative RT-PCR was performed as previously described.(6) The 25 μl reaction consisted of SYBR Green PCR Master Mix (PE Applied Biosystems, CA), rat specific primers for genes Igf2, Col1a2, Mmp9, Pthr1, Fmod, Agc1 at a concentration of 300 nM, and 5 ng pooled cDNA. All samples were run in triplicate along with 18srRNA as reference gene (forward, 5′GGTCATAAGCTTGCGTTGAT3′, reverse, 5′ TCAAGTTCGACCGTCTTCTC3′) and no template controls.

Analysis of Changes in Gene Expression

To identify differentially expressed genes in RZ, PZ, HZ and PC, RAE230 2.0 GeneChip microarray chips were used and Affymetrix GCOS/MAS 5 was used to generate a list of genes designated as “present” in any or all zones. Then, GeneSpring GX (Agilent Technologies,Palo Alto, CA) was used to identify differentially expressed genes in right zonal samples which were normalized to the left tibia at each of the three time points using the Robust Multiarray Analysis (RMA) method. An intensity filter of 10 times the lowest background expression level was applied in GeneSpring to normalized data in order to cut out very low expressed genes. Meaningful differential expression was determined at the 99.9% confidence level.(7) We included genes at the extreme tails of the distribution curve at +/- 3.3 z-scores from the mean, rather than an arbitrary fold difference.

Two additional steps were used for the microarray analysis: clustering self-organizing maps (SOM) and pathway analysis. Gene lists were clustered by SOM in GeneSpring, and clusters fitting the hypothesized temporal expression pattern (upregulation at 7 days, then decreasing expression at 11 and 16 days) were selected within each zone (RZ, PZ, HZ, and PC) for additional pathway analysis. Based on our hypothesis that the pattern of importance was limited to those genes that were significantly differentially expressed at 7 days on the irradiated side compared to the non-irradiated side and then decreased, we selected either a 4×3 or a 3×2 matrix depending upon the best fit of the data. For the RZ there were 23 genes that passed our expression level filter and for PC there were only 18 genes that passed the expression level filter, so a smaller (3×2) matrix sufficed. By contrast, for the PZ and HZ, with 244 and 245 genes passing the expression level filter, respectively, a 4×3 matrix did not adequately separate out a limited number of clusters that fit the desired pattern. Therefore, we discarded the SOM in lieu of simply analyzing these genes as one large group, for PZ and HZ separately, for the purposes of pathway analysis.

Functional pathway analysis for all zones was accomplished using hypergeometric p-values, manually performed in Microsoft Excel, to confirm significance, accepting hypergeometric p

values of less than 0.05. In addition to the hypergeometric p-values, pathways also had to include >1 probe set from the experimental data set and to have a fold enrichment score (FER) ≥5 in order to be considered meaningful.

For all four growth plate zones (RZ, PZ, PC, HZ), overall pathway analysis of all genes that met the expression level filter was accomplished independent of their temporal expression pattern. As in the pathway analysis of the individual clusters, enrichment of GO functional groups was determined to be meaningful with the number of probe sets in our experimental data of >1, hypergeometric p-values of less than 0.05, and fold enrichment of ≥5. We also identified enriched GO groups that are involved in skeletal development and bone remodeling. The latter screen was determined based on a search utilizing AmiGO, a search engine for the GeneOntology database (http://amigo.geneontology.org/cgi-bin/amigo/go.cgi). The experimental series of data files has been deposited into the Gene Expression Omnibus (GEO) at NCBI (accession GSE9537).

Results

Of the 31,099 probe sets arrayed on the chips, 23 RZ genes, 244 PZ genes, 245 HZ genes, and 18 PC genes having differential expression within the previously defined parameters were selected for further analysis.

Reserve Zone

A 3×2 SOM was performed to cluster RZ genes, and three of the six clusters showed progressive upregulation from 7 to 11 to 16 days.(Supplementary Fig 1) Two clusters (1,2) and (1,3) fit the pattern of significant upregulation on day 7 with expression decreasing on days 11 and 16.(Supplementary Fig 1) On pathway analysis of this cluster, no significant enrichment of GO groups was determined. Cluster (1,3) comprised 6 genes of which 4 were significantly upregulated at seven days.(Table I)

On pathway analysis of the entire group of 23 RZ genes meeting the differential expression level filters, enrichment was seen for 16 GO ontology pathways, including 4 molecular and 12 biological but no cellular pathways. Hence, these represent enriched RZ pathways containing genes differentially expressed at some point during the period after irradiation, but not necessarily following the hypothesized time pattern. Of these, there were no pathways specifically involving BCMSD. (Table II) None of these 16 pathways had five or more probe sets in our experimental data.(Table III)

Proliferative Zone

For the PZ genes, both 3×2 and 4×3 SOM's were examined in cluster analysis, and all but one cluster followed to some extent the hypothesized pattern.(Supplementary Fig 2) Therefore, pathway analysis of the entire group of 244 genes passing the differential expression level filter was done in lieu of detailed cluster analysis.(Table IV) This showed 103 enriched pathways with a minimum of 2 probe sets and a minimum fold enrichment of 5. These pathways included 32 molecular, 28 cellular, and 43 biological pathways. Sixteen pathways (16%) involved BCMSD.(Table III) Forty-three of the 103 (42%) pathways had a minimum of five probe sets in our data set. Nine (9%) of those 103 pathways involved BCMSD and also had a minimum five probe sets in our data set.(Table III)

Perichondrium

For the PC genes, all clusters from the 3×2 SOM showed the trend of hypothesized importance. (Supplementary Fig 3) Therefore, pathway analysis of the entire group of 18 PC genes passing the differential expression level filter was done.(Table V) Pathway analysis for all 18 PC genes

passing the expression level filter showed enrichment of 52 pathways with a minimum of 2 probe sets per pathway from our data set. Of those 52, there were 5 molecular, 20 cellular, and 27 biological. (Table VI) Only 15 pathways (19%) involved 5 or more probe sets from our data, and 4 pathways (8%) were involved in BCMSD. However, none of those 4 pathways involving BCMSD involved 5 or more probe sets from our data.(Table III)

Hypertrophic Zone

For the HZ genes, all clusters from the 4×3 SOM showed the temporal trend of hypothesized importance.(Supplementary Fig 4) Therefore pathway analysis of the entire group of 245 HZ genes was done.(Table VII) For pathway analysis of all 245 HZ genes meeting the expression level filter, 201 pathways showed enrichment with a minimum 2 probe sets per pathway. Of these, there were 39 molecular, 21 cellular, and 141 biological pathways. Nineteen pathways (9%) involved BCMSD. (Table VII) Seventy-two pathways (36%) involved 5 or more probe sets from the current data set. There were 12 pathways (6%) that involved BCMSD as well as including a minimum of a 5 probe sets.(Table III)

Other overlapping and unique pathways not related to BCMSD are shown in Supplementary Table I for all zones. Gene Ontology trees for significantly enriched GO terms have been created using QuikGO (http://www.ebi.ac.uk/ego/) and can be found in supplementary Figures 5-8 for RZ, PZ, PC and HZ, respectively.

Real Time Results

To confirm the microarray results, real-time quantitative RT-PCR was performed with a set of rat specific primers and template cDNA generated by reverse-transcription PCR. The primers were designed to selected genes among the 18 genes listed in Table V and 37 genes in Table VIII that were determined to show significant early upregulation by microarray filters. These six genes included insulin-like growth factor 2 (Igf2), procollagen type I alpha 2 (Col1a2), matrix metallopeptidase 9 (Mmp9), parathyroid hormone receptor 1 (Pthr1), fibromodulin (Fmod), and aggrecan 1 (Agc1). Real time PCR LOG 2 ratios (R/L) of two genes, Igf2 and Col1a2, from irradiated (R) and non-irradiated (L) perichondrium over 7, 11 and 16 days showed significant early upregulation followed by later downregulation., this real time PCR data highly correlated with the microarray results (R>0.99, Table IX-A). Additionally, four genes, Mmp9, Pthr1, Fmod and Agc1, indicated significant early upregulated changes at 7 days in HZ and PZ. The LOG 2 ratios (R/L) of these genes were also validated with real time PCR, in which high correlation coefficients between the microarray and real time PCR data were present. (Table IX-B)

Discussion

The damaging effect of radiotherapy on growth plate chondrocytes is well documented and has been the focus of much laboratory evaluation to date. (4,8-18) Since radiotherapy is sometimes necessary for the treatment of musculoskeletal oncology tumors in skeletally immature patients, previous work has focused on the beneficial effects of radioprotectants in selectively preventing growth plate damage. (4,8-18) However, the chondroprotective effects of these drugs are incomplete. Recent interest in our laboratory has focused on the use of selective radiorecovery agents to stimulate growth plate return without stimulating tumor growth. Animal models suggest that the irradiated growth plate has some capacity to recover after injury. (10,12,16) The appearance of chondrocyte clones which repopulate the growth plate and account in large part for its functional recovery are preceded by the early upregulation of specific genes. (10,12,16,17,18) We hypothesized that the genes and pathways of greatest importance to growth plate recovery are upregulated early on during the temporal growth plate recovery process. (17,18) In this experiment, laser microdissection was accomplished on four

growth plate zones (RZ, PC, PZ and HZ) following a clinically relevant fractionated 17.5 Gy (5 × 3.5 Gy) irradiation. The purpose was to identify individual genes and pathways showing early upregulation that may play an important role in growth plate radiorecovery. Based upon the results, we can now confirm our hypotheses that (1) differential upregulation of specific gene expression exists between irradiated and nonirradiated RZ, PZ, PC and HZ chondrocytes and (2) that a number of factors potentially vital to growth plate recovery do follow a pattern of early upregulation followed by a decrease in expression.

Each zone examined in the current study had a number of pathways showing enrichment following the pattern of hypothesized importance to growth plate recovery, yet few met the strictest filters applied. Among the four zones, the majority of the clusters for the PC, PZ, and HZ fit the hypothesized pattern, whereas only half of the RZ clusters did. The PZ and HZ were the two zones with the greatest number of genes showing a 10 fold change at 7 days after initiation of fractionated irradiation, having 244 and 245 genes, respectively, meeting those criteria. These two zones also showed enrichment of the most functional pathways involved BCMSD (9 in PZ and 12 in HZ) at the strictest filter criteria (having a minimum of five transcripts). Broadening the filter to include a minimum of only two transcripts, the PZ showed 16 and the HZ showed 19 enriched pathways involved BCMSD. In using the broadened filter criteria the PC also showed four enriched pathways BCMSD. With only 18 PC genes and 23 RZ genes having passed the differential expression level filter, it is not surprising that none of the pathways involved in BCMSD had a minimum of five transcripts in either of those two zones.

There was considerable overlap between the 9 PZ and 12 HZ BCMSD pathways showing enrichment in the PZ and HZ. The 9 overlapping pathways included: 4 involved in skeletal development (skeletal development, ossification, bone remodeling and cartilage development) and 5 involved in extracellular matrix or extracellular matrix structural constituent (extracellular matrix structural constituent, proteinaceous extracellular matrix, collagen, extracellular matrix, and extracellular matrix part). Because of some overlap in GO terminology, some of the skeletal development pathways were also involved in bone remodeling and cartilage development. (Table III)

Earlier analogous work in our laboratory with post-irradiation laser microdissection utilized a more pragmatic but less clinically applicable radiation scheme of a single 17.5 Gy fraction in examining the early post-radiation growth plate response. (17,18) In those studies, microarray analysis was performed on chondrocytes obtained by laser microdissection from the proliferative zone (PZ) and hypertrophic zone (HZ) of normal and irradiated tibia growth plates. Furthermore, pathway analysis was not as rigorous as in the current report. From the perspective of cytokines and growth factors in the earlier work, IGF2 was found to be up-regulated in the PZ and CTGF to be up-regulated in both the PZ and HZ at one week after single fraction irradiation.(17) Since this earliest time point examined occurred prior to the histomorphometric appearance of growth plate recovery previously reported in this immature animal radiation model, IGF2 and CTGF were suggested to be important to early growth plate recovery.(10,17) By two weeks after that single 17.5 Gy fraction, a number of other growth factors and cytokines (CTGF and Pthr1 in both zones, CXCL12 and its receptor in the PZ, and IL-17b and bone morphogenetic protein 2 in the HZ) showed upregulation, suggesting a possible later role in radiorecovery.(17)

Three of the growth factor and cytokine genes previously reported to be upregulated were found to fit the hypothesized pattern of early upregulation after fractionated radiation. These genes were also involved in the 12 enriched pathways shared by the proliferative and hypertrophic zones. These include PTHr1 in both zones, CTGF in HZ, and BMP2 in HZ. While each of these genes were significantly upregulated 7 days after the initiation of the fractionation

scheme, only CTGF had followed that pattern in the earlier work of single fraction irradiation, while both PTHr1 and BMP2 were elevated later in the response sequence. Interestingly, CTGF remained elevated at both timepoints in our earlier work, but while it remained meaningfully upregulated through 11 days after this fractionation scheme, it was no longer upregulated by 16 days. The other potentially important early growth factor contributor to growth plate recovery, IGF2, was not among the 34 genes following the hypothesized pattern drawn from the 12 enriched pathways. (Table VIII) Putting these findings together, two explanations seem plausible. First, IGF2 may play less of a role in recovery from the smaller fractionated radiotherapy insults while BMP2 and PTHR1, along with CTGF, may play more of a role. The alternative explanation is that a very early peak of IGF2 and CTGF was missed with the peak in CTGF persisting longer.

From the perspective of extracellular matrix (ECM) changes after irradiation, the report of Zhang et al utilized the same single 17.5 Gy fraction with laser microdissection of only the PZ and HZ in preparation for microarray, real-time PCR, and in situ hybridization. (18) In that work, both at one and two weeks after irradiation, normally expressed ECM genes and others not highly expressed in the normal growth plate showed upregulation. Conversely, metalloproteinases and cathepsins were down-regulated. Premature terminal differentiation was observed in the PZ by gene expression changes resulting in features of the normal HZ. In addition to normally expressed ECM genes (such as Ibsp, Mgp, Col1a2, Col1a1), ECM genes not highly expressed in the normal growth plate in the earlier study included several members of the small leucine-rich proteins (SLRP) and the ezrin-radixin-moesin (ERM) family. The accumulation of matrix following radiation injury to the growth plate, as previously reported in a predominately histomorphometric study, correlated well with the observed changes in ECM gene expression in the single fraction model.(10,18) Upregulation of the less commonly described growth plate genes was offered as support for their importance in the injury and repair response.(18)

Among the list of 34 genes fitting the hypothesized temporal pattern of importance and derived from the genes comprising the 12 enhanced PZ and/or HZ pathways, there were nine extracellular matrix genes that had previously been reported as being meaningfully upregulated at some point during the recovery phases after growth plate irradiation.(18) Matrix Gla protein and fibronectin followed a similar pattern as reported previously, being meaningfully upregulated at 7 days after the initiation of the fractionated scheme but then also remaining elevated throughout the time of observation in both zones. Fibromodulin also showed a similar pattern as it had following the single fraction irradiation, remaining upregulated in both zones for longer than just one week after initiating fractionated irradiation. Secreted acidic cysteine rich glycoprotein was upregulated early after irradiation in both manuscripts, but only within the HZ in the current results. Conversely, procollagen type I alpha 2 was meaningfully upregulated in both zones early after irradiation in the current manuscript but was similarly upregulated only in the PZ after single fraction irradiation. Aggrecan was upregulated (only in the HZ) in the current manuscript but not in the previously reported one. Interestingly, two proteases that had been downregulated early after irradiation by single fraction irradiation (cathepsin K and tissue inhibitor of metalloproteinase 2) were upregulated early on after the current scheme along with other proteases, including matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 3.

A number of pathways that are potential targets for manipulation of the post-radiation growth plate recovery process have been identified. Some of the genes involved in these pathways are identical to those identified as being important in this process using an earlier model. Still, many do not have a previously defined role in radiorecovery response or even the growth plate as a whole in some cases. Further work will be needed to determine the precise roles that each

of these pathways has and how they may be manipulated to bring about selective radiorecovery responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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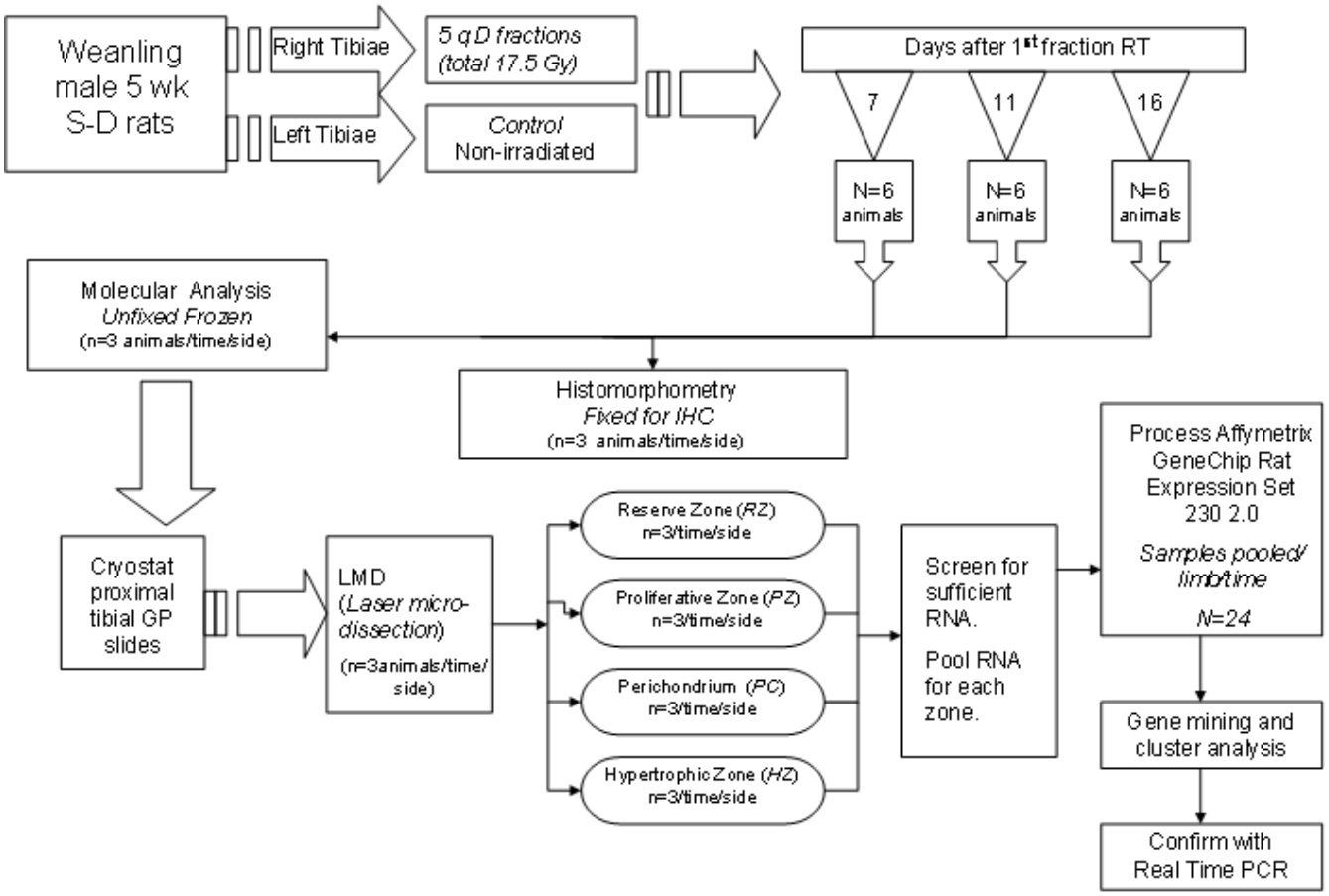

Figure 1.
Flow chart of experimental methods and sample organization for microarray analysis.

Table I

Fold changes of irradiated RZ chondrocytes normalized to non-irradiated RZ chondrocytes over time from cluster (1,3). Red indicates significantly upregulated fold changes while green indicates significantly downregulated fold changes

Differential Expression of RZ genes in Cluster (1,3)							
			99.9% CI				
			Log2 ratio (R/L)				
			7d	11d	16d		
		Mean Log2	0.00	0.01	0.01		
		StDev	0.75	0.44	0.72		
		+99.9 CL	2.53	1.49	2.44		
		# Incr 99.9CL	73	119	315		
		-99.9 CL	-2.53	-1.47	-2.41		
		# Decr 99.9CL	371	280	93		
			Log2 ratio (R/L)			Fold Change	
Systematic Name	Gene Title	Gene Symbol	7days	11days	16days	7days	11days
1393160_at	Transcribed locus, weakly similar to NP_035665.2 T-box 3 protein isoform 1 [Mus musculus]	---	3.68	0.44	0.16	12.83	1.36
1377558_at	Cell division cycle 25 homolog A (S. cerevisiae)	Cdc25a	3.68	0.48	-0.73	12.80	1.39
1376938_at	Protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform	Ppp2r2a	3.62	-0.44	-0.55	12.28	-1.35
1380747_at	Pre-B-cell leukemia transcription factor 1 (predicted)	Pbx1_predicted	3.48	0.84	0.07	11.16	1.80
1368655_at	proteoglycan peptide core protein	Pgsg	2.29	-3.25	-3.31	4.89	-9.52
1377998_at	coproporphyrinogen oxidase	Cpox	1.07	-3.57	-0.96	2.11	-11.92
				Falls within +99.9% CI			Fold Change
				Falls within -99.9% CI			Fold Change

Table II

RZ enriched pathways from the cluster analysis showing hypergeometric p values less than 0.05. These pathways were derived from the only RZ cluster showing differential upregulation at 7 days (compared to non-irradiated chondrocytes) followed by a decrease at 11 and 16 days

RZ Enriched Pathways after Differential Expression Analysis of 23 Genes in Gene List

GO Parent Category	GO Term	GO (Child) Term	GO ID	% of Array	% of list	Fold Enrichment	P value	Total Probe Sets On Array	Probe Sets in Your List
Biological Process	Biological Regulation	positive regulation of cell proliferation	8284	1.16%	8.70%	7.49	0.026658	360	2
	Cell Cycle	mitosis	7067	0.65%	8.70%	13.48	0.009159	200	2
		M phase of mitotic cell cycle	87	0.65%	8.70%	13.28	0.009417	203	2
		M phase	279	0.84%	8.70%	10.37	0.014874	260	2
		mitotic cell cycle	278	1.14%	8.70%	7.64	0.025753	353	2
		cell cycle phase	22403	1.19%	8.70%	7.29	0.027969	370	2
	Cellular Process	protein amino acid dephosphorylation	6470	0.60%	8.70%	14.49	0.007994	186	2
		cellular morphogenesis during differentiation	904	1.14%	8.70%	7.64	0.025753	353	2
		cell proliferation	8283	3.33%	17.39%	5.23	0.005678	1031	4
	Metabolic Process	dephosphorylation	16311	0.67%	8.70%	13.02	0.009766	207	2
	Multicellular Organismal Process	sensory perception	7600	1.20%	8.70%	7.27	0.028102	371	2
	Response to Stimulus	response to drug	42493	0.69%	8.70%	12.66	0.010299	213	2
Molecular Function	Catalytic Activity	phosphoprotein phosphatase activity	4721	0.69%	8.70%	12.54	0.010480	215	2
		phosphoric monoester hydrolase activity	16791	1.09%	8.70%	8.00	0.023727	337	2
		phosphoric ester hydrolase activity	42578	1.40%	8.70%	6.21	0.036843	434	2
	Enzyme Regulator Activity	GTPase regulator activity	30695	1.42%	8.70%	6.13	0.037714	440	2

Table III

Enriched pathways (independent of time course) related to bone, cartilage, matrix and/or skeletal development (BCMSD) comprised of 5 or more probe sets from our data and their corresponding zones

Ontologies associated with Bone, Cartilage, Matrix and Skeletal Development with 5 or more probe sets form our data									
		GO Term Associations							
Go ID	GO Term	Skeletal Development	Bone Remodeling	Bone Mineralization	Extracellular matrix	Extracellular matrix structural constituent	Cartilage development	GP Zone	
1501	skeletal development	√						PZ	HZ
1503	ossification	√	√					PZ	HZ
5201	extracellular matrix structural constituent					√		PZ	HZ
5578	proteinaceous extracellular matrix				√			PZ	HZ
5581	collagen				√			PZ	HZ
5604	basement membrane				√				HZ
30282	bone mineralization	√	√	√					HZ
31012	extracellular matrix				√			PZ	HZ
44420	extracellular matrix part				√			PZ	HZ
45453	bone resorption	√	√						HZ
46849	bone remodeling	√	√					PZ	HZ
51216	cartilage development	√					√	PZ	HZ

Table IV

PZ 103 enriched pathways from the cluster analysis showing hypergeometric p values less than 0.05. These pathways were derived from the entire PZ gene list showing differential upregulation at 7 days (compared to non-irradiated chondrocytes) followed by a decrease at 11 and 16 days. Gray indicates a pathways association with BCMSD

PZ Enriched Pathways after Differential Expression Analysis of 244 Genes in Gene List

GO Parent Category	GO Term	GO (Child) Term	GO ID	% of Array	% of list	Fold Enrichment	P value	Total Probe Sets On Array	Probe Sets in Your List
Biological Process	Biological Regulation/Cellular Process	iron ion homeostasis	6879	0.11%	1.23%	11.55	0.002079	33	3
		transition metal ion homeostasis	46916	0.18%	1.64%	9.07	0.000918	56	4
	Biological Regulation/Multicellular Organismal Process	regulation of sensory perception of pain	51930	0.03%	0.82%	25.41	0.002608	10	2
		regulation of sensory perception	51931	0.03%	0.82%	25.41	0.002608	10	2
	Biological Regulation/Multicellular Organismal Process	bone resorption	45453	0.13%	1.23%	9.77	0.003323	39	3
	Cellular Process	collagen fibril organization	30199	0.05%	1.23%	23.82	0.000244	16	3
		extracellular matrix organization and biogenesis	30198	0.25%	2.05%	8.36	0.000309	76	5
	Developmental Process/Multicellular Organismal Process	cartilage development	51216	0.18%	2.46%	13.37	0.000005	57	6
		bone mineralization	30282	0.13%	1.23%	9.77	0.003323	39	3
		ossification	1503	0.59%	4.92%	8.33	0.000000	183	12
		biomineral formation	31214	0.59%	4.92%	8.33	0.000000	183	12
		skeletal development	1501	1.09%	8.20%	7.54	0.000000	337	20
		tissue development	9888	1.52%	7.79%	5.11	0.000000	472	19
	Developmental Process/Multicellular Organismal Process/Cellular Process/ Biological Adhesion	cartilage condensation	1502	0.06%	1.23%	19.06	0.000481	20	3
	Localization	iron ion transport	6826	0.12%	1.64%	14.12	0.000172	36	4
		transition metal ion transport	41	0.23%	1.64%	7.26	0.002055	70	4
		phosphate transport	6817	0.35%	2.05%	5.88	0.001454	108	5

PZ Enriched Pathways after Differential Expression Analysis of 244 Genes in Gene List

GO Parent Category	GO Term	GO (Child) Term	GO ID	% of Array	% of list	Fold Enrichment	P value	Total Probe Sets On Array	Probe Sets in Your List
	Metabolic Process	biopolymer biosynthetic process	43284	0.25%	1.64%	6.43	0.003139	79	4
		carbohydrate catabolic process	16052	0.41%	2.46%	5.96	0.000473	128	6
	Metabolic Process/Cellular Process	translational elongation	6414	0.13%	1.64%	12.40	0.000284	41	4
		glycolysis	6096	0.22%	2.46%	11.05	0.000016	69	6
		nicotinamide metabolic process	6769	0.11%	1.23%	10.89	0.002456	35	3
		pyridine nucleotide metabolic process	19362	0.12%	1.23%	10.03	0.003092	38	3
		glucose catabolic process	6007	0.27%	2.46%	9.07	0.000050	84	6
		hexose catabolic process	19320	0.29%	2.46%	8.47	0.000073	90	6
		monosaccharide catabolic process	46365	0.29%	2.46%	8.47	0.000073	90	6
		alcohol catabolic process	46164	0.30%	2.46%	8.20	0.000087	93	6
		water-soluble vitamin metabolic process	6767	0.25%	1.64%	6.60	0.002872	77	4
		oxygen and reactive oxygen species metabolic process	6800	0.60%	3.69%	6.11	0.000016	187	9
		cellular carbohydrate catabolic process	44275	0.40%	2.46%	6.10	0.000418	125	6
		glucose metabolic process	6006	0.55%	3.28%	5.94	0.000058	171	8
		group transfer coenzyme metabolic process	6752	0.28%	1.64%	5.84	0.004369	87	4
		ATP metabolic process	46034	0.29%	1.64%	5.71	0.004719	89	4
		translation	6412	1.91%	10.25%	5.37	0.000000	591	25
	Multicellular Organismal Process	tissue remodeling	48771	0.75%	6.56%	8.76	0.000000	232	16
		bone remodeling	46849	0.68%	5.74%	8.47	0.000000	210	14
	Obsolete Biological Process	tricarboxylic acid cycle intermediate metabolic process	6100	0.09%	1.23%	13.14	0.001437	29	3
	Response to Stimulus	response to carbohydrate stimulus	9743	0.10%	1.23%	12.70	0.001584	30	3
		response to hydrogen peroxide	42542	0.14%	1.23%	9.07	0.004078	42	3

PZ Enriched Pathways after Differential Expression Analysis of 244 Genes in Gene List

GO Parent Category	GO Term	GO (Child) Term	GO ID	% of Array	% of list	Fold Enrichment	P value	Total Probe Sets On Array	Probe Sets in Your List
		response to mechanical stimulus	9612	0.19%	1.64%	8.47	0.001181	60	4
		response to estrogen stimulus	43627	0.25%	1.64%	6.60	0.002872	77	4
		response to oxidative stress	6979	0.52%	2.87%	5.52	0.000256	161	7
		response to steroid hormone stimulus	48545	0.47%	2.46%	5.19	0.000951	147	6
Cellular Component	Cell	cytosolic ribosome (sensu Eukaryota)	5830	0.23%	5.74%	25.05	0.000000	71	14
		cytosolic part	44445	0.49%	8.20%	16.83	0.000000	151	20
		extrinsic to membrane	19898	0.36%	2.05%	5.72	0.001634	111	5
		cytosol	5829	2.52%	13.52%	5.36	0.000000	782	33
	Cell/Macromolecular Complex	phosphopyruvate hydratase complex	15	0.01%	0.82%	63.52	0.000364	4	2
		eukaryotic 48S initiation complex	16283	0.10%	3.69%	36.89	0.000000	31	9
		proton-transporting ATP synthase complex, catalytic core F(1)	45261	0.03%	0.82%	31.76	0.001648	8	2
		proton-transporting two-sector ATPase complex, catalytic domain	33178	0.03%	0.82%	25.41	0.002608	10	2
		eukaryotic 43S preinitiation complex	16282	0.15%	3.69%	24.33	0.000000	47	9
		hemoglobin complex	5833	0.04%	0.82%	21.17	0.003765	12	2
		proteasome core complex (sensu Eukaryota)	5839	0.07%	1.23%	18.15	0.000557	21	3
		proteasome complex (sensu Eukaryota)	502	0.17%	1.64%	9.77	0.000698	52	4
	Cell/Macromolecular Complex/Organelle	small ribosomal subunit	15935	0.17%	3.69%	21.17	0.000000	54	9
		cytosolic large ribosomal subunit (sensu Eukaryota)	5842	0.12%	2.05%	17.17	0.000010	37	5
		large ribosomal subunit	15934	0.20%	2.05%	10.25	0.000121	62	5
	Cell/Organelle	rough endoplasmic reticulum membrane	30867	0.03%	0.82%	28.23	0.002103	9	2
	Cell/Organelle/Envelope	mitochondrial respiratory chain	5746	0.12%	1.23%	10.59	0.002658	36	3

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PZ Enriched Pathways after Differential Expression Analysis of 244 Genes in Gene List

GO Parent Category	GO Term	GO (Child) Term	GO ID	% of Array	% of list	Fold Enrichment	P value	Total Probe Sets On Array	Probe Sets in Your List
		mitochondrial membrane part	44455	0.21%	1.64%	7.82	0.001577	65	4
	Extracellular Matrix	extracellular matrix	31012	0.92%	8.20%	8.92	0.000000	285	20
		extracellular matrix part	44420	0.43%	3.69%	8.53	0.000001	134	9
	Extracellular Matrix/Extracellular Region	collagen type I	5584	0.01%	0.82%	63.52	0.000364	4	2
		fibrillar collagen	5583	0.06%	1.64%	28.23	0.000010	18	4
		collagen	5581	0.18%	2.05%	11.34	0.000075	56	5
		basement membrane	5604	0.26%	1.64%	6.27	0.003422	81	4
	Extracellular Region/Extracellular Matrix	proteinaceous extracellular matrix	5578	0.90%	8.20%	9.07	0.000000	280	20
	Organelle	cytosolic small ribosomal subunit (sensu Eukaryota)	5843	0.10%	3.69%	36.89	0.000000	31	9
		ribosome	5840	0.87%	7.79%	8.97	0.000000	269	19
	Organelle/Macromolecular Complex	ribonucleoprotein complex	30529	1.59%	9.02%	5.66	0.000000	494	22
Molecular Function	Antioxidant Activity	antioxidant activity	16209	0.23%	1.64%	7.06	0.002270	72	4
	Binding	organic acid binding	43177	0.01%	0.82%	84.70	0.000184	3	2
		rRNA binding	19843	0.06%	2.05%	31.76	0.000000	20	5
		calcium-dependent protein binding	48306	0.04%	0.82%	23.10	0.003162	11	2
		phosphatidylinositol-4,5-bisphosphate binding	5546	0.04%	0.82%	19.55	0.004415	13	2
		collagen binding	5518	0.07%	1.23%	18.15	0.000557	21	3
		calcium-dependent phospholipid binding	5544	0.11%	1.64%	14.52	0.000154	35	4
		hyaluronic acid binding	5540	0.10%	1.23%	12.30	0.001740	31	3
		NAD binding	51287	0.14%	1.64%	11.55	0.000372	44	4
		copper ion binding	5507	0.35%	2.46%	7.06	0.000195	108	6

PZ Enriched Pathways after Differential Expression Analysis of 244 Genes in Gene List

GO Parent Category	GO Term	GO (Child) Term	GO ID	% of Array	% of list	Fold Enrichment	P value	Total Probe Sets On Array	Probe Sets in Your List
	Catalytic Activity	phosphopyruvate hydratase activity	4634	0.01%	0.82%	63.52	0.000364	4	2
		aldehyde-lyase activity	16832	0.02%	0.82%	50.82	0.000603	5	2
		procollagen-proline dioxygenase activity	19798	0.02%	0.82%	36.30	0.001246	7	2
		peptidyl-proline dioxygenase activity	31543	0.02%	0.82%	36.30	0.001246	7	2
		oxidoreductase activity, acting on heme group of donors, oxygen as acceptor	16676	0.08%	1.64%	21.17	0.000034	24	4
		oxidoreductase activity, acting on heme group of donors	16675	0.08%	1.64%	21.17	0.000034	24	4
		heme-copper terminal oxidase activity	15002	0.08%	1.64%	21.17	0.000034	24	4
		protein-lysine 6-oxidase activity	4720	0.04%	0.82%	21.17	0.003765	12	2
		threonine endopeptidase activity	4298	0.07%	1.23%	18.15	0.000557	21	3
		oxidoreductase activity, acting on peroxide as acceptor	16684	0.17%	1.64%	9.77	0.000698	52	4
	Catalytic Activity/Antioxidant Activity	peroxiredoxin activity	51920	0.03%	0.82%	31.76	0.001648	8	2
		thioredoxin peroxidase activity	8379	0.03%	0.82%	31.76	0.001648	8	2
		peroxidase activity	4601	0.17%	1.64%	9.77	0.000698	52	4
		cytochrome-c oxidase activity	4129	0.08%	1.64%	21.17	0.000034	24	4
	Enzyme Regulator Activity	phospholipase inhibitor activity	4859	0.05%	1.23%	22.42	0.000294	17	3
	Structural Molecule Activity	structural constituent of bone	8147	0.03%	1.23%	47.64	0.000026	8	3
		extracellular matrix structural constituent	5201	0.33%	4.51%	13.84	0.000000	101	11
		structural constituent of ribosome	3735	0.80%	7.79%	9.77	0.000000	247	19
		structural molecule activity	5198	2.59%	13.93%	5.37	0.000000	804	34
	Transporter Activity	oxygen transporter activity	5344	0.04%	0.82%	19.55	0.004415	13	2
		hydrogen ion transporter activity	15078	0.40%	2.87%	7.17	0.000053	124	7
		monovalent inorganic cation transporter activity	15077	0.45%	2.87%	6.35	0.000111	140	7

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Table V

Fold changes of 18 genes isolated from irradiated PC chondrocytes normalized to non-irradiated PC chondrocytes over time within the 52 significantly enhanced pathways shown in Table VI. Red indicates significantly upregulated fold changes while green indicates significantly downregulated fold changes. Note that all of the genes showed the predicted pattern of early upregulation followed by progressive decrease

Differential Expression of 18 genes in the PC which follow our hypothesized pattern								
			99.9% CI					
			Log2 ratio (R/L)					
			7d	11d	16d			
		Mean Log2	0.01	0.00	-0.01			
		StDev	0.54	0.41	0.45			
		+99.9 CL	1.83	1.37	1.51			
		# Incr 99.9CL	209	73	132			
		-99.9 CL	-1.82	-1.38	-1.53			
		# Decr 99.9CL	279	201	270			
			Log2 ratio (R/L)			Fold Change		
Probe ID	Gene Title	Gene Symbol	7d	11d	16d	7days	11days	16days
1398882_at	ribosomal protein S5	Rps5	4.85	-0.18	-1.28	28.80	-1.13	-2.43
1398324_at	similar to 60S ribosomal protein L18a	MGC72957	4.57	-0.24	-1.39	23.75	-1.18	-2.62
1371307_at	ribosomal protein, large, P1	Rplp1	4.27	-0.71	-2.20	19.24	-1.64	-4.60
1367721_at	syndecan 4	Sdc4	4.21	-0.45	-1.16	18.50	-1.37	-2.24
1367595_s_at	beta-2 microglobulin	B2m	4.12	-0.99	-1.10	17.36	-1.99	-2.14
1367635_at	prolyl 4-hydroxylase, beta polypeptide	P4hb	3.95	0.48	-1.09	15.42	1.39	-2.13
1393240_at	EGF-containing fibulin-like extracellular matrix protein 2	Efemp2	3.94	0.36	-2.38	15.30	1.28	-5.21
1370341_at	enolase 2, gamma	Eno2	3.92	1.53	0.16	15.09	2.89	1.11
1392171_at	chitinase 3-like 1	Chi3l1	3.90	0.07	-1.28	14.93	1.05	-2.43
1367571_a_at	insulin-like growth factor 2	Igf2	3.72	-0.03	-0.98	13.15	-1.02	-1.97
1371305_at	ribosomal protein L8	Rpl8	3.68	0.29	-0.23	12.79	1.22	-1.17
1375066_at	similar to RIKEN cDNA 6330512M04 gene (predicted)	RGD1563319_predicted	3.61	-0.50	-1.02	12.21	-1.42	-2.03
1377472_at	Transcribed locus		3.61	-0.02	-1.02	12.18	-1.02	-2.03
1367569_at	ribosomal protein SA	Rpsa	3.50	0.32	-2.44	11.28	1.25	-5.42
1367560_at	acidic ribosomal phosphoprotein P0	Arbp	3.48	-0.13	-2.31	11.16	-1.09	-4.96

Differential Expression of 18 genes in the PC which follow our hypothesized pattern

1369113_at	gremlin 1 homolog, cysteine knot superfamily (Xenopus laevis)	Grem1	1.88	0.30	-3.92	3.69	1.23	-15.17
1370155_at	procollagen, type I, alpha 2	Col1a2	1.75	-0.78	-3.42	3.37	-1.71	-10.67
1375001_at	Transcribed locus		1.27	0.28	-5.59	2.42	1.21	-48.28
1373829_at	Transcribed locus		0.78	-0.49	-3.99	1.71	-1.40	-15.86
				Falls within +99.9% CI			Fold Change +2	
				Falls within +99.9% CI			Fold Change -2	

Table VI

PC 52 enriched pathways from the differential expression analysis showing hypergeometric p values less than 0.05. These pathways were derived from the entire PC gene list showing differential upregulation at 7 days (compared to non-irradiated chondrocytes) followed by a decrease at 11 and 16 days. Gray indicates a pathways association with BCMSD

PC Enriched Pathways after Differential Expression Analysis of 18 Genes in Gene List

GO Parent Category	GO Term	GO (Child)Term	GO ID	% of Array	% of list	Fold Enrichment	P value	Total Probe Sets On Array	Probe Sets in Your List
Biological Process	Biological Regulation	regulation of cell size	8361	0.70%	11.11%	15.87	0.006676	217	2
		regulation of biological quality	65008	2.94%	16.67%	5.68	0.013177	910	3
		cell growth	16049	0.69%	11.11%	16.10	0.006502	214	2
	Cellular Process	transmembrane receptor protein tyrosine kinase signaling pathway	7169	0.96%	16.67%	17.34	0.000622	298	3
		enzyme linked receptor protein signaling pathway	7167	1.37%	16.67%	12.19	0.001689	424	3
		cell-cell signaling	7267	3.27%	16.67%	5.10	0.017314	1014	3
	Developmental Process	morphogenesis of an epithelium	2009	0.48%	11.11%	22.96	0.003296	150	2
	Developmental Process/Multicellular Organismal Process	embryonic morphogenesis	48598	0.61%	11.11%	18.32	0.005083	188	2
		skeletal development	1501	1.09%	16.67%	15.33	0.000883	337	3
		organ morphogenesis	9887	1.87%	16.67%	8.89	0.004030	581	3
		embryonic development	9790	1.42%	11.11%	7.81	0.024589	441	2
		organ development	48513	5.52%	27.78%	5.04	0.002085	1710	5
	Growth	growth	40007	1.34%	11.11%	8.32	0.021975	414	2
	Metabolic Process	biopolymer biosynthetic process	43284	0.25%	11.11%	43.60	0.000943	79	2
		carbohydrate catabolic process	16052	0.41%	11.11%	26.91	0.002425	128	2
		carbohydrate metabolic process	5975	1.81%	11.11%	6.13	0.037495	562	2
		nitrogen compound metabolic process	6807	2.00%	11.11%	5.56	0.044269	620	2
		macromolecule biosynthetic process	9059	2.96%	33.33%	11.26	0.000009	918	6
		macromolecule catabolic process	9057	1.50%	11.11%	7.42	0.026897	464	2

PC Enriched Pathways after Differential Expression Analysis of 18 Genes in Gene List

GO Parent Category	GO Term	GO (Child)Term	GO ID	% of Array	% of list	Fold Enrichment	P value	Total Probe Sets On Array	Probe Sets in Your List
		biosynthetic process	9058	5.41%	33.33%	6.16	0.000238	1678	6
	Metabolic Process/Cellular Process	translational elongation	6414	0.13%	11.11%	84.01	0.000256	41	2
		cellular carbohydrate catabolic process	44275	0.40%	11.11%	27.56	0.002316	125	2
		translation	6412	1.91%	33.33%	17.48	0.000001	591	6
		cellular macromolecule catabolic process	44265	1.20%	11.11%	9.23	0.018220	373	2
		cellular carbohydrate metabolic process	44262	1.36%	11.11%	8.16	0.022738	422	2
		amine metabolic process	9308	1.87%	11.11%	5.95	0.039445	579	2
		cellular catabolic process	44248	2.09%	11.11%	5.31	0.047774	649	2
Cellular Component	Cell	cytosolic part	44445	0.49%	27.78%	57.03	0.000000	151	5
		cell surface	9986	0.80%	11.11%	13.95	0.008520	247	2
		cytosol	5829	2.52%	27.78%	11.01	0.000062	782	5
	Cell/Macromolecular Process	eukaryotic 48S initiation complex	16283	0.10%	11.11%	111.11	0.000146	31	2
		eukaryotic 43S preinitiation complex	16282	0.15%	11.11%	73.29	0.000336	47	2
		ribonucleoprotein complex	30529	1.59%	33.33%	20.92	0.000000	494	6
	Extracellular Matrix	extracellular matrix	31012	0.92%	16.67%	18.13	0.000547	285	3
	Extracellular Region	extracellular region part	44421	6.97%	44.44%	6.37	0.000012	2162	8
		extracellular region	5576	7.58%	44.44%	5.86	0.000022	2351	8
		extracellular space	5615	6.65%	38.89%	5.85	0.000085	2062	7
	Extracellular	proteinaceous extracellular matrix	5578	0.90%	16.67%	18.45	0.000520	280	3
	Macromolecular Complex	macromolecular complex	32991	8.61%	44.44%	5.16	0.000053	2669	8
	Organelle	non-membrane-bound organelle	43228	5.76%	33.33%	5.79	0.000330	1785	6

PC Enriched Pathways after Differential Expression Analysis of 18 Genes in Gene List

GO Parent Category	GO Term	GO (Child)Term	GO ID	% of Array	% of list	Fold Enrichment	P value	Total Probe Sets On Array	Probe Sets in Your List
	Organelle/Cell	intracellular non-membrane-bound organelle	43232	5.76%	33.33%	5.79	0.000330	1785	6
	Organelle/Cell/Macromolecular Complex	cytosolic small ribosomal subunit (sensu Eukaryota)	5843	0.10%	11.11%	111.11	0.000146	31	2
		cytosolic ribosome (sensu Eukaryota)	5830	0.23%	22.22%	97.03	0.000000	71	4
		cytosolic large ribosomal subunit (sensu Eukaryota)	5842	0.12%	11.11%	93.09	0.000208	37	2
		small ribosomal subunit	15935	0.17%	11.11%	63.79	0.000444	54	2
		large ribosomal subunit	15934	0.20%	11.11%	55.56	0.000584	62	2
		ribosome	5840	0.87%	33.33%	38.41	0.000000	269	6
Molecular Function	Binding	RNA binding	3723	2.32%	16.67%	7.19	0.007141	719	3
	Molecular Transducer	transmembrane receptor activity	4888	3.22%	16.67%	5.18	0.016598	997	3
	Structural Molecule Activity	structural constituent of ribosome	3735	0.80%	33.33%	41.84	0.000000	247	6
		extracellular matrix structural constituent	5201	0.33%	11.11%	34.10	0.001528	101	2
		structural molecule activity	5198	2.59%	44.44%	17.14	0.000000	804	8

Table VII

HZ 201 enriched pathways from differential expression analysis showing hypergeometric p values less than 0.05. These pathways were derived from the 245 HZ genes showing differential upregulation at 7 days (compared to non-irradiated chondrocytes) followed by a decrease at 11 and 16 days. Gray indicates a pathways association with BCMSD

HZ Enriched Pathways after Differential Expression Analysis of 245 Genes in Gene List

GO Parent Category	GO Term	GO (Child)Term	GO ID	% of Array	% of list	Fold Enrichment	P value	Total Probe Sets On Array	Probe Sets in Your List
Biological Process	Biological Process/Developmental Process/ Multicellular Organismal Process/Cellular Process	regulation of dendrite development	50773	0.07%	0.82%	11.00	0.013340	23	2
		negative regulation of neurogenesis	50768	0.10%	0.82%	8.16	0.023022	31	2
		positive regulation of neurogenesis	50769	0.15%	1.22%	7.91	0.005926	48	3
		regulation of neurogenesis	50767	0.33%	2.04%	6.26	0.001110	101	5
	Biological Regulation	regulation of cell size	8361	0.70%	3.67%	5.25	0.000052	217	9
		negative regulation of protein kinase activity	6469	0.24%	1.22%	5.06	0.018716	75	3
	Biological Regulation/Celluilar Process	negative regulation of cyclin-dependent protein kinase activity	45736	0.04%	0.82%	23.01	0.003187	11	2
		negative regulation of progression through cell cycle	45786	0.53%	2.86%	5.37	0.000303	165	7
		negative regulation of Ras protein signal transduction	46580	0.04%	0.82%	23.01	0.003187	11	2
		negative regulation of small GTPase mediated signal transduction	51058	0.04%	0.82%	19.47	0.004450	13	2
		regulation of cyclin-dependent protein kinase activity	79	0.15%	1.22%	8.44	0.004978	45	3
		iron ion homeostasis	6879	0.11%	0.82%	7.67	0.025732	33	2
		positive regulation of mononuclear cell proliferation	32946	0.11%	0.82%	7.67	0.025732	33	2
		regulation of progression through mitotic cell cycle	7346	0.13%	0.82%	6.33	0.035973	40	2
	Biological Regulation/Cellular Process/Growth	cell growth	16049	0.69%	3.67%	5.32	0.000047	214	9
	Biological Regulation/Cellular Process/Immune System Process	positive regulation of lymphocyte proliferation	50671	0.11%	0.82%	7.67	0.025732	33	2

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	Biological Regulation/cellular Process/Localization	positive regulation of cell migration	30335	0.12%	1.22%	9.99	0.003126	38	3
		positive regulation of cell motility	51272	0.15%	1.22%	8.44	0.004978	45	3
	Biological Regulation/Developmental Process	negative regulation of developmental process	51093	0.38%	2.04%	5.36	0.002148	118	5
	Biological Regulation/Developmental Process/ Multicellular Organismal Process	regulation of bone mineralization	30500	0.07%	0.82%	11.50	0.012277	22	2
	Biological Regulation/Localization/Immune System Process/Cellular Process	positive regulation of leukocyte migration	2687	0.01%	0.82%	126.53	0.000062	2	2
		regulation of leukocyte migration	2685	0.01%	0.82%	63.27	0.000367	4	2
	Biological Regulation/Locomotion	positive regulation of locomotion	40017	0.15%	1.22%	8.44	0.004978	45	3
	Biological Regulation/Metabolic Process/Cellular Process	activation of NF-kappaB transcription factor	51092	0.06%	0.82%	13.32	0.009305	19	2
		positive regulation of transcription factor activity	51091	0.09%	0.82%	9.37	0.017934	27	2
		regulation of transcription factor activity	51090	0.15%	0.82%	5.62	0.043895	45	2
	Biological Regulation/Multicellular Organismal Process	bone resorption	45453	0.13%	2.45%	19.47	0.000001	39	6
		multicellular organismal homeostasis	48871	0.15%	2.45%	15.82	0.000002	48	6
		positive regulation of bone remodeling	46852	0.07%	0.82%	11.50	0.012277	22	2
		regulation of bone remodeling	46850	0.21%	1.22%	5.93	0.012590	64	3
		hemostasis	7599	0.35%	2.04%	5.75	0.001600	110	5
	Biological Regulation/Multicellular Organismal Process/Developmental Process	regulation of ossification	30278	0.17%	1.22%	7.03	0.008108	54	3

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	Biological Regulation/MulticellularOrganismal Process	tissue homeostasis	1894	0.15%	2.45%	15.82	0.000002	48	6
	Bioogical Regulatio/Cellular Process/Developmental Process	negative regulation of cell differentiation	45596	0.28%	1.63%	5.89	0.004259	86	4
	Cellular Process	collagen fibril organization	30199	0.05%	2.04%	39.54	0.000000	16	5
		elastic fiber assembly	48251	0.02%	0.82%	36.15	0.001256	7	2
		extracellular matrix organization and biogenesis	30198	0.25%	5.31%	21.64	0.000000	76	13
		extracellular structure organization and biogenesis	43062	0.53%	5.31%	9.97	0.000000	165	13
		ER-nuclear signaling pathway	6984	0.09%	0.82%	9.04	0.019162	28	2
		epithelial cell proliferation	50673	0.18%	1.22%	6.78	0.008920	56	3
		transforming growth factor beta receptor signaling pathway	7179	0.26%	1.63%	6.33	0.003325	80	4
	Cellular Process/Biological Adhesion	cell-matrix adhesion	7160	0.32%	2.04%	6.39	0.001018	99	5
		cell-substrate adhesion	31589	0.34%	2.04%	6.08	0.001259	104	5
	Cellular Process/Biological Adhesion/Developmental Process/Multicellular Organismal Process	cartilage condensation	1502	0.06%	1.63%	25.31	0.000016	20	4
	Cellular Process/Biological Regulation	positive regulation of epithelial cell proliferation	50679	0.09%	0.82%	9.37	0.017934	27	2
		regulation of epithelial cell proliferation	50678	0.14%	0.82%	5.75	0.042275	44	2
	Cellular Process/Localization	protein targeting to membrane	6612	0.10%	0.82%	7.91	0.024364	32	2
	Cellular Process/Metabolic Process	peptide cross-linking	18149	0.05%	0.82%	18.08	0.005151	14	2
		proteoglycan biosynthetic process	30166	0.07%	0.82%	11.00	0.013340	23	2

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		sphingolipid biosynthetic process	30148	0.08%	0.82%	10.54	0.014439	24	2
		purine nucleoside triphosphate biosynthetic process	9145	0.23%	2.04%	9.04	0.000216	70	5
		ribonucleoside triphosphate biosynthetic process	9201	0.23%	2.04%	9.04	0.000216	70	5
		purine ribonucleoside triphosphate biosynthetic process	9206	0.23%	2.04%	9.04	0.000216	70	5
		nucleoside triphosphate biosynthetic process	9142	0.24%	2.04%	8.55	0.000279	74	5
		ATP metabolic process	46034	0.29%	2.45%	8.53	0.000070	89	6
		actin filament polymerization	30041	0.15%	1.22%	7.91	0.005926	48	3
		ribonucleoside triphosphate metabolic process	9199	0.32%	2.45%	7.75	0.000118	98	6
		purine ribonucleoside triphosphate metabolic process	9205	0.32%	2.45%	7.75	0.000118	98	6
		purine nucleoside triphosphate metabolic process	9144	0.32%	2.45%	7.67	0.000125	99	6
		nucleoside triphosphate metabolic process	9141	0.34%	2.45%	7.16	0.000180	106	6
		purine ribonucleotide biosynthetic process	9152	0.31%	2.04%	6.52	0.000932	97	5
		actin filament depolymerization	30042	0.13%	0.82%	6.49	0.034445	39	2
		purine ribonucleotide metabolic process	9150	0.41%	2.45%	5.98	0.000464	127	6
		ribonucleotide biosynthetic process	9260	0.34%	2.04%	5.97	0.001366	106	5
		hexose catabolic process	19320	0.29%	1.63%	5.62	0.004967	90	4
		monosaccharide catabolic process	46365	0.29%	1.63%	5.62	0.004967	90	4
		actin polymerization and/or depolymerization	8154	0.30%	1.63%	5.44	0.005544	93	4
		alcohol catabolic process	46164	0.30%	1.63%	5.44	0.005544	93	4
		ribonucleotide metabolic process	9259	0.45%	2.45%	5.38	0.000788	141	6
		coenzyme biosynthetic process	9108	0.40%	2.04%	5.10	0.002638	124	5
	Cellular Process/Response to Stimulus	unfolded protein response	30968	0.06%	0.82%	12.65	0.010258	20	2

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	Developmental Process	aging	7568	0.20%	1.22%	6.22	0.011135	61	3
	Developmental Process/Multicellular Organismal Process	bone mineralization	30282	0.13%	2.04%	16.22	0.000013	39	5
		cartilage development	51216	0.18%	2.45%	13.32	0.000006	57	6
		ossification	1503	0.59%	4.49%	7.61	0.000000	183	11
		biomineral formation	31214	0.59%	4.49%	7.61	0.000000	183	11
		skeletal development	1501	1.09%	7.35%	6.76	0.000000	337	18
		ureteric bud development	1657	0.15%	0.82%	5.50	0.045529	46	2
	Developmental Process/Multicellular Organismal Process/Biological Regulation/Cellular Process	regulation of dendrite morphogenesis	48814	0.05%	0.82%	14.89	0.007518	17	2
	Developmental Process/Multicellular Organismal Process/Cellular Process	dendrite morphogenesis	48813	0.07%	0.82%	11.50	0.012277	22	2
	Developmental Process/Multicellular Organismal Process/Cellular Process/Immune System Process	macrophage differentiation	30225	0.05%	0.82%	14.89	0.007518	17	2
	Developmental Process/Multicellular Organismal Process/Multicellular Organismal Process	osteoblast differentiation	1649	0.17%	1.63%	9.73	0.000708	52	4
	Localization	long-chain fatty acid transport	15909	0.04%	0.82%	19.47	0.004450	13	2
		fatty acid transport	15908	0.06%	0.82%	12.65	0.010258	20	2
		energy coupled proton transport, down electrochemical gradient	15985	0.18%	2.04%	11.30	0.000076	56	5
		iron ion transport	6826	0.12%	1.22%	10.54	0.002688	36	3
		phosphate transport	6817	0.35%	3.27%	9.37	0.000002	108	8
		proton transport	15992	0.35%	2.45%	6.90	0.000219	110	6
		hydrogen transport	6818	0.37%	2.45%	6.66	0.000265	114	6
		transition metal ion transport	41	0.23%	1.22%	5.42	0.015780	70	3

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	Localization/Cellular Process/Immune System Process	leukocyte migration	50900	0.18%	1.63%	9.04	0.000932	56	4
	Localization/Cellular Process/Metabolic Process	ATP synthesis coupled proton transport	15986	0.18%	2.04%	11.30	0.000076	56	5
	Metabolic Process	sequestering of lipid	19915	0.03%	0.82%	25.31	0.002629	10	2
		collagen metabolic process	32963	0.05%	0.82%	18.08	0.005151	14	2
		multicellular organismal macromolecule metabolic process	44259	0.05%	0.82%	18.08	0.005151	14	2
		multicellular organismal macromolecule catabolic process	44266	0.05%	0.82%	18.08	0.005151	14	2
		multicellular organismal protein metabolic process	44268	0.05%	0.82%	18.08	0.005151	14	2
		peptidoglycan metabolic process	270	0.06%	0.82%	13.32	0.009305	19	2
		protein tetramerization	51262	0.09%	0.82%	9.37	0.017934	27	2
	Metabolic Process/Cellular Process	nucleoside phosphate metabolic process	6753	0.20%	2.04%	10.37	0.000114	61	5
		ATP biosynthetic process	6754	0.20%	2.04%	10.37	0.000114	61	5
		N-acetylglucosamine metabolic process	6044	0.08%	0.82%	9.73	0.016737	26	2
		glutathione metabolic process	6749	0.08%	0.82%	9.73	0.016737	26	2
		glucosamine metabolic process	6041	0.09%	0.82%	9.04	0.019162	28	2
		proteasomal ubiquitin-dependent protein catabolic process	43161	0.09%	0.82%	9.04	0.019162	28	2
		oxidative phosphorylation	6119	0.29%	2.45%	8.34	0.000079	91	6
		amino sugar metabolic process	6040	0.10%	0.82%	8.16	0.023022	31	2
		proteoglycan metabolic process	6029	0.10%	0.82%	7.91	0.024364	32	2
		glycolysis	6096	0.22%	1.63%	7.34	0.001981	69	4
		group transfer coenzyme metabolic process	6752	0.28%	2.04%	7.27	0.000578	87	5
		glucose catabolic process	6007	0.27%	1.63%	6.03	0.003931	84	4

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		purine nucleotide biosynthetic process	6164	0.35%	2.04%	5.86	0.001480	108	5
		sphingolipid metabolic process	6665	0.22%	1.22%	5.58	0.014676	68	3
		purine nucleotide metabolic process	6163	0.45%	2.45%	5.46	0.000734	139	6
		oxygen and reactive oxygen species metabolic process	6800	0.60%	3.27%	5.41	0.000109	187	8
		cellular respiration	45333	0.15%	0.82%	5.27	0.048844	48	2
	Metabolic Process/Multicellular Organismal Process	collagen catabolic process	30574	0.05%	0.82%	18.08	0.005151	14	2
		multicellular organismal protein catabolic process	44254	0.05%	0.82%	18.08	0.005151	14	2
		multicellular organismal catabolic process	44243	0.05%	0.82%	16.87	0.005896	15	2
		multicellular organismal metabolic process	44236	0.07%	0.82%	12.05	0.011249	21	2
	Multicellular Organismal Process	protein digestion	44256	0.05%	0.82%	18.08	0.005151	14	2
		bone remodeling	46849	0.68%	5.71%	8.44	0.000000	210	14
		tissue remodeling	48771	0.75%	6.12%	8.18	0.000000	232	15
		coagulation	50817	0.34%	2.04%	6.08	0.001259	104	5
	Obsolete Biological Process	tricarboxylic acid cycle intermediate metabolic process	6100	0.09%	0.82%	8.73	0.020420	29	2
	Reproduction/Multicellular Organismal Process/ Developmental Process/Multi-organism Process	embryo implantation	7566	0.08%	0.82%	10.12	0.015571	25	2
	Response to Stimulus	response to hexose stimulus	9746	0.08%	1.63%	20.24	0.000041	25	4
	Response to Stimulus	response to glucose stimulus	9749	0.08%	1.63%	20.24	0.000041	25	4
		response to carbohydrate stimulus	9743	0.10%	1.63%	16.87	0.000085	30	4
		response to organic cyclic substance	14070	0.07%	0.82%	12.05	0.011249	21	2

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		response to glucocorticoid stimulus	51384	0.14%	1.63%	12.05	0.000317	42	4
		response to steroid hormone stimulus	48545	0.47%	4.08%	8.61	0.000000	147	10
		wound healing	42060	0.70%	4.49%	6.38	0.000001	218	11
		acute-phase response	6953	0.13%	0.82%	6.17	0.037521	41	2
		response to hormone stimulus	9725	0.73%	4.49%	6.16	0.000002	226	11
		response to hydrogen peroxide	42542	0.14%	0.82%	6.03	0.039088	42	2
		response to drug	42493	0.69%	4.08%	5.94	0.000007	213	10
		response to oxidative stress	6979	0.52%	2.86%	5.50	0.000262	161	7
		response to organic substance	10033	0.55%	2.86%	5.18	0.000373	171	7
	Response to Stimulus/Biologocal Regulation/ Multicellular Organismal Process	blood coagulation	7596	0.33%	2.04%	6.14	0.001208	103	5
	Response to Stimulus/Localization/Immune System Process/Cellular Process	leukocyte chemotaxis	30595	0.13%	0.82%	6.17	0.037521	41	2
Cellular Component	Cell	lipid particle	5811	0.02%	0.82%	36.15	0.001256	7	2
		ER-Golgi intermediate compartment	5793	0.09%	1.22%	13.56	0.001313	28	3
		sarcoplasmic reticulum	16529	0.11%	0.82%	7.67	0.025732	33	2
		sarcoplasm	16528	0.11%	0.82%	7.23	0.028544	35	2
		ruffle	1726	0.14%	0.82%	5.89	0.040673	43	2
		sarcolemma	42383	0.15%	0.82%	5.27	0.048844	48	2
	Cell/Macromolecular Complex	eukaryotic 48S initiation complex	16283	0.10%	1.22%	12.24	0.001760	31	3
		proton-transporting two-sector ATPase complex	16469	0.22%	2.04%	9.44	0.000176	67	5
		eukaryotic 43S preinitiation complex	16282	0.15%	1.22%	8.08	0.005600	47	3
	Cell/Organelle	integral to endoplasmic reticulum membrane	30176	0.18%	1.22%	6.90	0.008508	55	3

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		intrinsic to endoplasmic reticulum membrane	31227	0.20%	1.22%	6.22	0.011135	61	3
	Extracellular Matrix	extracellular matrix part	44420	0.43%	6.94%	16.05	0.000000	134	17
		extracellular matrix	31012	0.92%	11.43%	12.43	0.000000	285	28
	Extracellular Region/Extracellular Matrix	collagen	5581	0.18%	3.27%	18.08	0.000000	56	8
		basement membrane	5604	0.26%	4.49%	17.18	0.000000	81	11
		anchoring collagen	30934	0.05%	0.82%	15.82	0.006686	16	2
		proteinaceous extracellular matrix	5578	0.90%	11.43%	12.65	0.000000	280	28
		basal lamina	5605	0.11%	0.82%	7.23	0.028544	35	2
	Organelle/Macromolecular Complex/Cell	cytosolic small ribosomal subunit (sensu Eukaryota)	5843	0.10%	1.22%	12.24	0.001760	31	3
		cytosolic ribosome (sensu Eukaryota)	5830	0.23%	1.63%	7.13	0.002192	71	4
		small ribosomal subunit	15935	0.17%	1.22%	7.03	0.008108	54	3
Molecular Function	Antioxidant Activity	antioxidant activity	16209	0.23%	1.22%	5.27	0.016925	72	3
	Binding	S100 beta binding	48154	0.01%	0.82%	84.35	0.000185	3	2
		calcium-dependent protein binding	48306	0.04%	1.63%	46.01	0.000001	11	4
		IgE binding	19863	0.02%	0.82%	36.15	0.001256	7	2
		collagen binding	5518	0.07%	2.45%	36.15	0.000000	21	6
		S100 alpha binding	48155	0.03%	0.82%	31.63	0.001661	8	2
		GABA receptor binding	50811	0.03%	0.82%	28.12	0.002119	9	2
		phosphatidylinositol-4,5-bisphosphate binding	5546	0.04%	0.82%	19.47	0.004450	13	2
		immunoglobulin binding	19865	0.05%	0.82%	15.82	0.006686	16	2
		ferric iron binding	8199	0.06%	0.82%	14.06	0.008391	18	2
		calcium-dependent phospholipid binding	5544	0.11%	1.22%	10.85	0.002483	35	3
		integrin binding	5178	0.16%	1.63%	9.92	0.000659	51	4

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		selenium binding	8430	0.09%	0.82%	8.73	0.020420	29	2
		hyaluronic acid binding	5540	0.10%	0.82%	8.16	0.023022	31	2
		copper ion binding	5507	0.35%	2.45%	7.03	0.000199	108	6
		protein complex binding	32403	0.41%	2.45%	6.03	0.000445	126	6
		glycosaminoglycan binding	5539	0.40%	2.04%	5.06	0.002727	125	5
	Catalytic Activity	gelatinase B activity	4229	0.01%	0.82%	126.53	0.000062	2	2
		collagenase activity	8133	0.02%	0.82%	50.61	0.000608	5	2
		protein-lysine 6-oxidase activity	4720	0.04%	1.22%	31.63	0.000100	12	3
		oxidoreductase activity, acting on the CH-NH2 group of donors, oxygen as acceptor	16641	0.07%	1.22%	17.25	0.000647	22	3
		oxidoreductase activity, acting on the CH-NH2 group of donors	16638	0.09%	1.22%	14.06	0.001182	27	3
		oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors	16706	0.09%	0.82%	9.37	0.017934	27	2
	Catalytic Activity/Transporter Activity	hydrogen-exporting ATPase activity, phosphorylative mechanism	8553	0.10%	1.63%	16.33	0.000097	31	4
		cation-transporting ATPase activity	19829	0.29%	2.04%	7.03	0.000671	90	5
		ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism	15662	0.33%	1.63%	5.01	0.007282	101	4
	Enzyme Regulator Activity	metalloendopeptidase inhibitor activity	8191	0.03%	0.82%	25.31	0.002629	10	2
		phospholipase inhibitor activity	4859	0.05%	0.82%	14.89	0.007518	17	2
		endopeptidase inhibitor activity	4866	0.55%	2.86%	5.21	0.000360	170	7
		protease inhibitor activity	30414	0.55%	2.86%	5.18	0.000373	171	7
		enzyme inhibitor activity	4857	1.06%	5.31%	5.00	0.000002	329	13

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	Molecular Transducer Activity	secretin-like receptor activity	1633	0.12%	0.82%	6.66	0.032937	38	2
	Structural Molecule Activity	structural constituent of bone	8147	0.03%	0.82%	31.63	0.001661	8	2
		extracellular matrix structural constituent	5201	0.33%	4.49%	13.78	0.000000	101	11
	Transcription Regulator Activity	specific RNA polymerase II transcription factor activity	3704	0.14%	0.82%	5.75	0.042275	44	2
	Transporter Activity	hydrogen ion transporting ATPase activity, rotational mechanism	46961	0.18%	2.04%	11.10	0.000083	57	5
		hydrogen ion transporting ATP synthase activity, rotational mechanism	46933	0.17%	1.63%	9.37	0.000815	54	4
		hydrogen ion transporter activity	15078	0.40%	2.45%	6.12	0.000410	124	6
		monovalent inorganic cation transporter activity	15077	0.45%	2.45%	5.42	0.000760	140	6

Table VIII

Fold changes of 34 genes isolated from the 12 enriched pathways of Table III for PZ and HZ. Red indicates significantly upregulated fold changes while green indicates significantly downregulated fold changes. Note that all of the genes showed the predicted pattern of early upregulation followed by progressive decrease. * and † represent genes present in earlier single-fraction studies, references 16 and 17, respectively. NC represents genes with no significant change

Differentially expressed genes from within the 12 enriched pathways of Table III For PZ and HZ													
			Hypertophic Zone						**Proliferative Zone**					
			99.9% CI						99.9% CI					
			Log2 ratio (R/L)						Log2 ratio (R/L)					
			7d	11d	16d				7d	11d	16d			
		Mean Log2	0.05	-0.02	-0.01				0.04	-0.01	-0.01			
		StDev	0.89	0.60	0.52				0.80	0.46	0.38			
		+99.9 CL	3.07	2.02	1.74				2.73	1.53	1.26			
		# Incr 99.9CL	346	440	152				500	142	53			
		-99.9 CL	-2.97	-2.05	-1.75				-2.65	-1.55	-1.29			
		# Decr 99.9CL	441	41	238				29	135	310			
			HZ						PZ					
			Log2 ratio (R/L)			Fold Change			Log2 ratio (R/L)			Fold Change		
Probe ID	Gene Name	Symbol	7days	11days	16days	7days	11days	16days	7day	11day	16day	7day	11day	16day
1367568_a_at	matrix Gla protein	Mgp *	8.07	5.07	2.37	268.16	33.58	5.18	3.90	2.54	1.85	14.90	5.81	3.60
1370234_at	fibronectin 1	Fn1 *	6.27	2.98	1.53	77.15	7.90	2.89	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1369166_at	matrix metallopeptidase 9	Mmp9	6.13	0.83	-1.29	69.90	1.77	-2.45	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1371369_at	procollagen, type VI, alpha 2	Col6a2	5.57	0.46	-0.04	47.59	1.38	-1.03	3.47	0.41	0.13	11.08	1.33	1.09
1370259_a_at	parathyroid hormone receptor 1	Pthr1 †	5.52	-0.07	0.18	45.76	-1.05	1.13	5.34	-0.03	0.61	40.42	-1.02	1.53
1367584_at	annexin A2	Anxa2	5.27	0.59	-0.46	38.65	1.50	-1.37	4.39	-0.01	0.58	20.98	-1.01	1.49
1398270_at	bone morphogenetic protein 2	Bmp2 †	5.22	0.28	0.97	37.27	1.22	1.97	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1367942_at	acid phosphatase 5, tartrate resistant	Acp5	5.04	1.10	-2.16	32.86	2.15	-4.48	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1388618_at	nidogen 2	Nid2	5.02	0.36	-0.22	32.41	1.29	-1.17	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1367700_at	fibromodulin	Fmod *	4.69	0.53	-0.01	25.90	1.45	-1.01	4.36	1.09	-0.13	20.53	2.13	-1.10
1388332_at	Ras-related C3 botulinum toxin substrate 1	Rac1	4.68	2.25	-0.52	25.71	4.76	-1.43	4.66	-0.05	-0.24	25.24	-1.03	-1.18
1389836_a_at	Tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory)	Timp3	4.59	0.51	-0.15	24.06	1.42	-1.11	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1367563_at	secreted acidic cysteine rich glycoprotein	Sparc *	4.58	1.53	-0.18	23.84	2.89	-1.13	4.43	1.60	-0.07	21.56	3.03	-1.05

Differentially expressed genes from within the 12 enriched pathways of Table III For PZ and HZ													
1369947_at	cathepsin K	Ctsk *	4.55	1.67	-1.64	23.46	3.18	-3.13	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1370155_at	procollagen, type I, alpha 2	Col1a2 *	3.40	4.52	-3.21	10.55	23.00	-9.25	3.90	-0.62	1.22	14.91	-1.53	2.32
1367594_at	biglycan	Bgn	4.51	0.13	0.50	22.78	1.09	1.42	3.57	-0.29	-0.86	11.91	-1.22	-1.81
1393240_at	EGF-containing fibulin-like extracellular matrix protein 2	Efemp2	4.08	0.30	0.16	16.89	1.23	1.12	4.31	0.11	0.56	19.79	1.08	1.47
1368171_at	lysyl oxidase	Lox	4.15	1.03	-0.79	17.72	2.04	-1.73	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1387137_at	cartilage oligomeric matrix protein	Comp	3.98	0.17	2.14	15.75	1.12	4.41	4.13	-0.03	0.02	17.55	-1.02	1.02
1387039_at	glypican 1	Gpc1	4.12	0.06	0.05	17.44	1.04	1.04	3.88	0.60	0.52	14.76	1.52	1.44
1386879_at	lectin, galactose binding, soluble 3	Lgals3	4.11	0.42	0.73	17.22	1.34	1.66	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1371349_at	procollagen, type VI, alpha 1 (predicted)	Col6a1	3.98	1.94	0.22	15.78	3.83	1.17	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1373615_at	frizzled-related protein	Frzb	3.95	0.81	1.71	15.51	1.76	3.27	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1387797_at	RAB7, member RAS oncogene family	Rab7	3.76	1.14	-0.36	13.56	2.20	-1.29	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1367581_a_at	secreted phosphoprotein 1	Spp1	3.72	0.41	1.09	13.20	1.33	2.12	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1368187_at	glycoprotein (transmembrane) nmb	Gpnmb	3.64	0.84	-1.96	12.45	1.79	-3.90	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1386940_at	tissue inhibitor of metalloproteinase 2	Timp2 *	3.64	0.23	-0.82	12.45	1.17	-1.76	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1367631_at	connective tissue growth factor	Ctgf †	3.61	3.20	0.61	12.21	9.17	1.53	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1387355_at	aggrecan 1	Agc1 *	3.56	1.34	0.31	11.83	2.53	1.24	3.60	1.58	-0.20	12.13	2.99	-1.15
1389966_at	procollagen, type VI, alpha 3 (predicted)	Col6a3	3.58	1.65	0.24	11.99	3.13	1.18	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1373210_at	laminin, beta 1 (predicted)	Lamb1	3.56	1.11	-0.44	11.80	2.16	-1.35	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1388494_at	Procollagen, type IV, alpha 2 (predicted)	Col4a2	2.78	1.88	-2.56	6.88	3.69	-5.90	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1388459_at	procollagen, type XVIII, alpha 1	Col18a1	0.53	0.49	-2.79	1.44	1.40	-6.91	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
1368885_at	ectonucleoside triphosphate diphosphohydrolase 1	Entpd1	0.29	0.15	-2.75	1.22	1.11	-6.74	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*
				Falls within +99.9% CI			Fold Change +2			Falls within +99.9% CI			Fold Change +2	
				Falls within -99.9% CI			Fold Change -2			Falls within -99.9% CI			Fold Change -2	

Table IX (A)

LOG2 ratio (R/L) of microarray (MC) and real time PCR (PCR) between irradiated (R) and non-irradiated (L) perichondrium

Probe ID	Gene Title	Gene Symbol	7d		11d		16d		R
			MC	PCR	MC	PCR	MC	PCR	
1367571_a_at	insulin-like growth factor 2	Igf2	3.72	2.3 (0.72/0.88)	-0.03	-0.15 (1.57/0.10)	-0.98	-1.23 (0.42/0.49)	0.99386
1370155_at	procollagen, type I, alpha 2	Col1a2	1.75	1.1 (0.76/0.49)	-0.78	-0.73 (0.51/0.28)	-3.42	-3.55 (0.82/0.59)	0.99395

* Standard deviations of ΔCT for real time PCR (R/L) are listed in parentheses.

Table IX (B)

LOG2 ratio (R/L) of microarray (MC) and real time PCR (PCR) between irradiated (R) and non-irradiated (L) in HZ and PZ at 7 days

Probe ID	Gene Title	Gene Symbol	HZ		PZ	
			MC	PCR*	MC	PCR*
1369166_at	matrix metallopeptidase 9	Mmp9	6.13	4.88 (0.87/0.60)		
1370259_a_at	parathyroid homone receptor 1	Pthr1	5.52	6.75 (1.11/1.35)	5.34	6.3 (2.22/2.06)
1367700_at	fibromodulin	Fmod	4.69	3.4 (1.85/1.04)	4.36	3.81 (1.73/1.01)
1368836_a_at	aggrecan 1	Agc1	3.99	2.99 (0.67/1.19)	4.04	2.63 (0.42/0.63)
Correlation efficient factor R				0.7234		0.9966

*Standard deviations of ΔCT for real time PCR (R/L) are listed in parentheses.