Published in final edited form as:

Biochem Biophys Res Commun. 2007 November 3; 362(4): 940–945. doi:10.1016/j.bbrc.2007.08.077.

microRNAs and regeneration: *let-7* members as potential regulators of dedifferentiation in lens and inner ear hair cell regeneration of the adult newt

Panagiotis A. Tsonis^{a,*}, Mindy K. Call^a, Matthew W. Grogg^a, Maureen A. Sartor^b, Ruth R. Taylor^c, Andrew Forge^c, Robert Fyffe^d, Robert Goldenberg^e, Richard Cowper-Sallari^f, and Craig R. Tomlinson^g

^a Department of Biology and Center for Tissue Regeneration and Engineering, University of Dayton, Dayton, OH 45469-2320

^b Center for Environmental Genetics Dept. of Environmental Health, University of Cincinnati, Cincinnati, OH 4526

^c Centre for Auditory Research, University College London Ear Institute, London WC1X 8EE

^d Department of Neuroscience, Cell Biology & Physiology, Boonshoft School of Medicine, Wright State University, Dayton, OH 45459

^e Division of Otolaryngology, Boonshoft School of Medicine, Wright State University, Dayton, OH 45459

^f Department of Genetics, Dartmouth Hitchcock Medical Center, Norris Cotton Cancer Center, Rubin 634, Dartmouth College, Lebanon, NH 03756

^g Departments of Medicine and Pharmacology & Toxicology, Dartmouth Hitchcock Medical Center, Norris Cotton Cancer Center, Rubin 634, Dartmouth College, Lebanon, NH 03756

Keywords

miRNAs; lens; hair cells; regeneration; dedifferentiation; stem cells

Introduction

Among adult vertebrates, regeneration of body parts and organs is largely restricted to some salamanders [1,2]. These animals can regenerate many body parts including their limbs, hair cells of the inner ear and lateral line, tail (and spinal cord), eye retina and lens. They achieve this by the process of transdifferentiation of terminally differentiated cells at the site of the injury. During this process one cell type changes to another, by dedifferentiating [3–5]. It is conceivable that such an event may involve global changes in gene expression, implicating a large number of genes [6]. What could then be the regulators of changes of such magnitude?

To provide some answers to these questions we decided to look at the expression profiles of miRNAs during lens and inner ear hair cell regeneration. miRNAs are short RNAs (20–22 nt)

*Corresponding author. Fax: +1-937-2292021, E-mail address: E-mail: panagiotis.tsonis@notes.udayton.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

which have complementary nucleotide sequences in target mRNAs. By binding to these target sequences miRNAs inhibit protein synthesis [7,8]. Since one miRNA can have target sequences in hundreds of different mRNAs they have been thought to act when rapid global regulation is needed. For example, miR430 can clear hundreds of maternal RNAs during development [9]. Likewise, differentiation of stem cells is linked to distinct miRNA expression [10,11]. Recently, miRNA presence in neoblasts (stem cells in planaria) has been associated with regenerative ability in that invertebrate animal [12]. Based on this work we believe that miRNAs may be key regulators during transdifferentiation, a process that requires regulation of many tissue-specific genes in a short time. To test this hypothesis we performed a microarray analysis of miRNAs during lens regeneration and inner ear hair cell regeneration.

After lentectomy, the dorsal iris pigment epithelial cells (PECs) dedifferentiate and then differentiate to lens-forming cells. Interestingly, the same cells from the ventral iris do not contribute to the process of regeneration [3,4]. After hair cell death due to aminoglycoside antibiotic treatment hair cells are regenerated by transdifferentiation of the supporting cells [13]. This event is mediated by direct transition without cell proliferation. We decided to investigate two different events of regeneration in order to compare possible common regulators. Our results provide strong evidence of association of miRNA expression and regeneration and imply a novel mechanism that might regulate transdifferentiation and regeneration.

Materials and Methods

Microarray analysis

We used mirVana miRNA Bioarrays V2 microarray slides from Ambion (Austin, TX), which contain most of the known mouse and human miRNAs along with others predicted by Ambion. The arrays were probed with RNA isolated from intact dorsal and ventral irises (day 0) and from dorsal and ventral irises taken 8 days after lentectomy. This time was selected because at day 8 the tip of the dorsal iris undergoes the crucial events of dedifferentiation, which will eventually lead to the regeneration of the new lens [3,4]. We then compared differences in the expression between intact dorsal and ventral irises as well as between 8-day dorsal and ventral irises. For hair cell regeneration, we isolated the labyrinths comprising the auditory and vestibular sensory epithelia. After treatment with 2mM gentamicin for 48 hours, the labyrinths were cultured *in vitro*. We used RNA samples from day 0 (end of antibiotic treatment), day 2, 7 and 12. Regenerated hair cells appear after 18 days in culture, so the period that we selected represents the time window of recovery, when the cells undergo 'reprogramming' for transdifferentiation [13].

Data normalization and analysis

The data were analyzed to identify differentially expressed miRNAs between dorsal and ventral tissues (intact or at day-8 post-lentectomy) as well as miRNAs at different times during hair cell regeneration. Analyses were performed using R statistical software and the limma Bioconductor package [14]. Data normalization was performed in two steps for each microarray separately [15–17]. First, background adjusted intensities were log-transformed and the differences (R) and averages (A) of log-transformed values were calculated as $R = \log_2(X1) - \log_2(X2)$ and $A = [\log_2(X1) + \log_2(X2)]/2$, where X1 and X2 denote the Cy5 and Cy3 intensities after subtracting local backgrounds, respectively. Second, normalization was performed by fitting the array-specific local regression model of R as a function of A. Normalized log-intensities for the two channels were then calculated by adding half of the normalized ratio to A for the Cy5 channel and subtracting half of the normalized ratio from A for the Cy3 channel. The statistical analysis was performed for each time point comparison and for each gene separately by fitting the following Analysis of Variance (ANOVA) model

[18]: $Y_{ijk} = \bar{T} + A_i + S_j + C_k + \epsilon_{ijk}$, where Y_{ijk} corresponds to the normalized log-intensity on the i^{th} array, with the j^{th} treatment condition, and labeled with the k^{th} dye ($k = 1$ for Cy5, and 2 for Cy3). $\bar{\mu}$ is the overall mean log-intensity, A_i is the effect of the i^{th} array, S_j is the effect of the j^{th} treatment and C_k is the gene-specific effect of the k^{th} dye. Resulting t-statistics for each comparison were modified using an empirical Bayesian moderated-T method [14]. This method uses variance estimates from all genes to improve the variance estimates of each individual gene. Estimates of fold-change and False Discovery Rates (*FDR*) were calculated [19].

Expression analysis via QPCR

In order to validate the microarray data, we examined the expression of selected miRNAs via QPCR. RNA from dorsal and ventral irises as well as from treated inner ear sensory epithelia was isolated using TRIreagent (Molecular Research Center, Cincinnati, OH) following the manufacturer's instructions, with the exception that 2 volumes of ethanol were used instead of isopropanol at the precipitation step. QPCR was performed using Ambion's mirVana™ qRT-PCR miRNA detection kit and mirVana™ qRT-PCR Primer Sets for miRNAs. We examined expression of miR148a and *let-7*. For normalization, Ambion's mirVana™ qRT-PCR Primer Set for 5S rRNA was used. Real-time PCR was performed with iCycler (BioRad) and SYBR Green I fluorescent dye (Molecular Probes, Carlsbad, CA) [20,21].

Results and Discussion

Expression during lens regeneration

The microarray analysis revealed the regulation of several miRNAs between the intact dorsal and ventral irises and between the irises at day 8 of regeneration. For example, some miRNAs were more highly expressed in the intact dorsal iris while others were in the intact ventral irises. Similar regulation was observed in the 8-day irises as well (Table 1). For example a V/D fold change of 7.65 means that the levels in the ventral iris are 7.65 times more than in the dorsal. A negative sign would indicate the opposite, higher levels in the dorsal. It is interesting to note that miRNAs thought to be eye specific in mammals, such as miR184 are also found in our list [22]. Among others that have been found to be expressed during mouse eye development are: miR181, miR124, miR204, miR125 [22]. All these have been found in our microarray, indicating that our analysis is quite valid. Of interest are members of the *let-7* family. Many of them (*let-7a-g*; highlighted with red color in Table 1) are found in the miRNA list, having the same overall expression pattern and deserve further attention. They were found in higher levels in the intact dorsal iris, but they were down-regulated in the dorsal iris during dedifferentiation. These miRNAs have been associated with control of cell cycle, which is a crucial event during dedifferentiation [23] and with oncogenic transformation [24]. Some other miRNA show an interesting up-regulation in the ventral iris. For example, miR148 is elevated in the intact ventral iris as well as in the 8-day ventral iris when compared with its dorsal counterparts. This might indicate that such miRNAs could be negative regulators (repress regeneration-related genes in the ventral iris). The expression patterns of miR148 were also verified by QPCR (Fig. 1a). We also examined expression of *let7* and as it is shown in Fig.1b it was found to be expressed in the irises. We should draw attention here to the fact that the Ambion primers for *let7* can pick several different members and that in principle QPCR and microarray are different techniques. Nevertheless, the QPCR data on *let7* expression provide an independent verification of its expression in newt irises. In a separate study we have reported the cloning of most of these miRNAs, which as expected show identity in sequence with their mammalian counterparts [25].

Expression during hair cell regeneration

Several miRNAs were found to be regulated during the initial process of dedifferentiation (Tables 2–4). Interestingly, many of the regulated miRNAs have also been identified in another study during maturation in mice [26]. These miRNAs are very different than the ones identified during lens regeneration. The primary exception is the members of the *let-7* family (highlighted red in Table 4). These miRNAs showed significant reduction in their expression levels at day 12 after antibiotic treatment, a period, which is characterized by the early events of regeneration. The expression was also verified via QPCR (Fig. 1c; see above for discussion). It is interesting to note here that this pattern is very similar in both lens and hair cell regeneration. *Let-7* miRNAs are known to promote terminal differentiation, tumor suppression and re-entry to the cell cycle. Thus, their down-regulation might implicate them as regulators of dedifferentiation, a critical event for regeneration to occur. Their common expression in two different regeneration events in the newt may indicate that initiation of regeneration in different tissue involves common signals. This is of paramount importance in the field and for the first time alludes to a novel mechanism of vertebrate regeneration. These miRNAs may become indispensable tools to probe and understand the remarkable regenerative capabilities in salamanders and possibly allow extension in other animals. Since miRNAs are relatively short they can be easily transfected *in vivo* the same way as morpholinos [27]. As techniques to over-express or down-regulate miRNAs are becoming more advanced and available we will be able to delineate the function and role of these miRNAs in regeneration. Comparative studies with the axolotl (a lens regeneration-incompetent salamander) or even mouse might further allow us to understand why such regenerative capability is restricted.

Acknowledgements

This work was supported by NEI grant EY10540 and by a research contract from Wright State University to PAT and by RNID to RRT and AF.

References

1. Tsonis PA. Regeneration in vertebrates. *Develop Biol* 2000;221:273–284. [PubMed: 10790325]
2. Sanchez Alvarado S, Tsonis PA. Bridging the regeneration gap: genetic insights from diverse animal models. *Nat Rev Genet* 2006;7:873–884. [PubMed: 17047686]
3. Tsonis PA, Del Rio-Tsonis K. Lens and retina regeneration: transdifferentiation, stem cells and clinical applications. *Exp Eye Res* 2004;78:161–172. [PubMed: 14729349]
4. Del Rio-Tsonis K, Tsonis PA. Eye regeneration at the molecular age. *Dev Dyn* 2003;226:211–224. [PubMed: 12557200]
5. Grogg MW, Call MK, Okamoto M, Vergara MN, Del Rio-Tsonis K, Tsonis PA. BMP inhibition-driven regulation of six-3 underlies induction of newt lens regeneration. *Nature* 2005;438:858–862. [PubMed: 16341014]
6. Makarev E, Call MK, Grogg MW, Atkinson DL, Milash B, Odelberg SJ, Tsonis PA. Gene expression signatures in the newt irises during lens regeneration. *FEBS Lett* 2007;581(9):1865–1870. [PubMed: 17434491]
7. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;116:281–297. [PubMed: 14744438]
8. Ambros V. The functions of animal microRNAs. *Nature* 2004;431:350–355. [PubMed: 15372042]
9. Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ, Schier AF. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* 2006;312:75–79. [PubMed: 16484454]
10. Suh MR, Lee Y, Kim JY, Kim SK, Moon SH, Lee JY, Cha KY, Chung HM, Yoon HS, Moon SY, Kim VN, Kim KS. Human embryonic stem cells express a unique set of microRNAs. *Dev Biol* 2004;270:488–498. [PubMed: 15183728]

11. Hatfield SD, Shcherbata HR, Fischer KA, Nakahara K, Carthew RW, Ruohola-Baker H. Stem cell division is regulated by the microRNA pathway. *Nature* 2005;435:974–978. [PubMed: 15944714]
12. Palakodeti D, Smielewska M, Graveley BR. MicroRNAs from the Planarian *Schmidtea mediterranea*: a model system for stem cell biology. *RNA* 2006;12(9):1640–1649. [PubMed: 16849698]
13. Taylor RR, Forge A. Hair cell regeneration in sensory epithelia from the inner ear of a urodele amphibian. *J Comp Neurol* 2005;484(1):105–120.
14. Wettenhall JM, Smyth GK. limmaGUI: a graphical user interface for linear modeling of microarray data. *Bioinformatics* 2004;20(18):3705–3706. [PubMed: 15297296]
15. Guo J, Sartor M, Karyala S, Medvedovic M, Kann S, Puga A, Ryan P, Tomlinson CR. Expression of genes in the TGF-beta signaling pathway is significantly deregulated in smooth muscle cells from aorta of aryl hydrocarbon receptor knockout mice. *Toxicol Appl Pharmacol* 2004;194:79–89. [PubMed: 14728982]
16. Karyala S, Guo J, Sartor M, Medvedovic M, Kann S, Puga A, Ryan P, Tomlinson CR. Different global gene expression profiles in benzo[a]pyrene- and dioxin-treated vascular smooth muscle cells of AHR-knockout and wild-type mice. *Cardiovasc Toxicol* 2004;4:47–73. [PubMed: 15034205]
17. Sartor M, Schwanekamp J, Halbleib D, Mohamed I, Karyala S, Medvedovic M, Tomlinson CR. Microarray results improve significantly as hybridization approaches equilibrium. *Biotechniques* 2004;36:790–6. [PubMed: 15152598]
18. Dudoit S, Yang Y, Callow MJ, Speed TP. Statistical methods for identifying differentially expressed genes in replicated cDNA microarray experiments. *Statistica Sinica* 2002;12:111–139.
19. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society B* 1995;57:289–300.
20. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001;29:e45. [PubMed: 11328886]
21. Ramakers C, Ruijter JM, Deprez RH, Moorman AF. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neurosci Lett* 2003;339:62–66. [PubMed: 12618301]
22. Ryan DG, Oliveira-Fernandes M, Lavker RM. MicroRNAs of the mammalian eye display distinct and overlapping tissue specificity. *Mol Vis* 2006;12:1175–1184. [PubMed: 17102797]
23. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* 2000;403(6772):901–906. [PubMed: 10706289]
24. Mayr C, Hemann MT, Bartel DP. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. *Science* 2007;315(5818):1576–1579. [PubMed: 17322030]
25. Makarev E, Spence JR, Del Rio-Tsonis K, Tsonis PA. Identification of microRNAs and other small RNAs from the adult newt eye. *Mol Vis* 2006;12:1386–1391. [PubMed: 17149364]
26. Weston MD, Pierce ML, Rocha-Sanchez S, Beisel KW, Soukup GA. MicroRNA gene expression in the mouse inner ear. *Brain Res* 2006;1111:95–104. [PubMed: 16904081]
27. Madhavan M, Haynes TL, Frisch NC, Call MK, Minich CM, Tsonis PA, Del Rio-Tsonis K. The role of Pax-6 in lens regeneration. *Proc Natl Acad Sci USA* 2006;103:14848–14853. [PubMed: 17003134]

Figure 1.

Analysis of microRNAs expression by QPCR. a: miR148 during lens regeneration, b: *let-7* during lens regeneration, c: *let-7* during hair cell regeneration. The comparisons in a and b and between normal dorsal iris (ND), normal ventral irises (NV), dorsal irises during regeneration (RD) and ventral iris during regeneration (RV) (see text for explanation). In c expression is compared with the expression on day 0 (set as 1; see text). The values are the average of triplicates $\pm$ standard deviation.

Table 1

Microarray analysis in the irises

clone	Avg. intensity	Fold change V/D	Fold change 8d V/D	clone	Avg. intensity	Fold change V/D	Fold change 8dV/D
hsa_miR_148	156	7.65	1.90	rno_miR_327	213	-1.49	1.07
ambi_miR_7012	96	4.87	-1.07	mmu_miR_292	238	3.81	-1.10
ambi_miR_7075	96	-3.98	-2.65	ambi_miR_7016	105	1.71	1.04
Control_4	86	-4.44	-1.44	rno_miR_327	161	2.27	1.07
hsa_miR_181	76	-4.12	-1.20	ambi_miR_71	136	-1.50	1.03
hsa_miR_365	99	-3.24	1.40	hsa_miR_124	371	1.28	-1.11
hsa_miR_142	108	-2.77	1.15	hsa_miR_370	182	2.87	1.68
ambi_miR_7067	139	-3.49	-3.36	mmu_miR_202	286	-1.88	1.06
mmu_miR_202	321	-2.54	1.31	mmu_miR_291	200	-2.33	1.26
hsa_miR_204	479	-2.72	-1.04	mmu_miR_351	57	-1.18	-1.27
hsa_miR_198	324	-3.45	-1.05	Control_4	135	1.40	1.02
ambi_miR_7057	602	-1.51	1.12	hsa_miR_342	2204	1.17	1.05
hsa_miR_299	70	2.27	1.99	hsa_miR_141	96	1.16	-1.61
hsa_miR_320	326	-1.74	1.23	hsa_miR_26a	131	-1.57	4.43
Has_miR_200	101	-1.88	-1.67	hsa_miR_182	139	1.24	1.69
hsa_miR_125	258	-2.98	-1.59	ambi_miR_7065	1094	1.22	-1.49
hsa_miR_181	165	1.84	1.43	ambi_miR_7065	1666	-1.12	-1.49
ambi_miR_7030	255	-6.18	1.28	ambi_miR_7024	175	1.37	1.57
ambi_miR_7076	51	1.99	-3.52	ambi_miR_7073	47	1.89	-2.53
ambi_miR_7034	106	-2.04	2.71	hsa_miR_151	77	-1.26	-1.39
ambi_miR_7030	173	-2.16	2.40	Control_1	16576	1.06	1.03
ambi_miR_7089	115	2.45	-1.75	ambi_miR_7044	272	-1.59	-1.08
hsa_miR_370	166	-1.49	2.32	hsa_miR_342	2215	1.07	1.04
ambi_miR_7046	155	3.52	1.10	Control_1	20562	-1.06	-1.10
hsa_miR_320	581	-1.73	1.10	hsa_miR_122	297	1.08	1.92
ambi_miR_7002	78	1.75	-2.03	Control_1	16825	-1.04	1.04
ambi_miR_7044	236	-1.84	1.06	Control_1	16345	-1.04	1.03
hsa_miR_302	122	1.38	1.38	hsa_miR_184	270	-1.38	1.90
hsa_miR_182	173	-1.71	-1.47	Control_1	19687	-1.04	1.02

clone	Avg. intensity	Fold change V/D	Fold change 8d V/D	clone	Avg. intensity	Fold change V/D	Fold change 8dV/D
ambi_miR_7075	88	-3.35	1.50	hsa_miR_181	198	1.28	1.05
hsa_miR_30d	104	-1.66	-1.60	ambi_miR_7058	224	1.24	1.52
ambi_miR_7063	208	-1.97	3.78	Control_1	17267	1.04	1.03
hsa_let_7d	267	-2.25	1.20	Control_1	21345	-1.04	-1.02
hsa_let_7c	242	-2.14	2.11	Control_1	19884	1.03	-1.05
hsa_let_7e	111	-15.17	1.45	hsa_miR_31	108	1.36	-1.28
hsa_let_7g	138	-1.68	1.40	Control_1	20201	-1.03	-1.01
hsa_let_7a	291	-1.37	-1.00	Control_1	19507	1.03	1.01
hsa_let_7b	157	-1.42	3.15	mmu_miR_298	299	1.20	3.11
hsa_let_7f	176	-1.21	1.04	Control_1	15189	1.03	-1.18
hsa_miR_184	151	-2.31	1.31	Control_1	14283	1.03	-1.01
ambi_miR_7058	175	-2.48	1.97	Control_1	18926	-1.02	1.01
hsa_miR_198	705	-1.62	-1.18	mmu_miR_298	369	1.13	1.66
hsa_miR_95	161	-1.28	-1.28	hsa_miR_124	520	-1.01	-1.14
mmu_miR_106	125	3.63	-1.22	hsa_miR_130	135	1.02	2.10
ambi_miR_7057	502	-1.35	1.33				

Table 2

Microarray analysis during hair cell regeneration. Day 2 vs 0

Clone	Fold Change	Avg Int
mmu_miR_151	2.0674	396.0573
mmu_miR_129_3p	-1.7490	169.7252
hsa_miR_373	-1.7328	708.3516
hsa_miR_520h	-1.6025	238.2225
hsa_miR_28	1.5548	451.8614
ambi_miR_7067	-1.5471	364.4230
hsa_miR_450	1.5033	665.0543
hsa_miR_190	-1.5217	654.7506
hsa_miR_489	-1.5724	234.2624
mmu_miR_330	-1.4925	139.4994

Table 3

Microarray analysis during hair cell regeneration. Day 7 vs 0

Clone	Fold Change	Avg. Int.	Clone	Fold Change	Avg. Int.
hsa_miR_95	-3.9855	1735.3084	hsa_miR_132	1.3824	362.8796
mmu_miR_155	-3.1804	20.5176	hsa_miR_489	1.3779	234.5939
hsa_miR_23a	-2.1884	84.5907	hsa_miR_296	1.3765	237.7511
mmu_miR_192	1.9234	54.6014	hsa_miR_188	-1.3726	671.5538
hsa_miR_20a	1.8358	142.0789	hsa_miR_202_AS	-1.3666	559.6628
hsa_miR_526b_AS	1.7350	188.8813	hsa_miR_526c	1.3663	402.3559
hsa_miR_126_AS	1.6630	245.0694	hsa_miR_125a	-1.3606	330.3474
rno_miR_297	-1.6628	688.0986	mmu_miR_322	-1.3596	204.0268
hsa_miR_127	-1.6426	352.7881	hsa_miR_302b_AS	1.3544	454.3263
hsa_miR_25	1.6124	234.1699	ambi_miR_7036	-1.3529	395.6788
hsa_miR_151	1.6029	354.0169	hsa_miR_126	-1.3513	367.3735
hsa_miR_450	-1.5850	549.2480	hsa_let_7a	1.3509	648.9146
hsa_miR_382	-1.5823	275.2389	hsa_let_7e	1.3499	692.5730
hsa_miR_208	-1.5805	182.8891	hsa_miR_21	1.3496	536.7623
hsa_miR_23a	-1.5549	95.2843	hsa_miR_24	-1.3487	344.4468
hsa_miR_196a	-1.5247	316.6415	hsa_miR_182_AS	1.3452	455.7878
hsa_miR_34a	-1.5161	668.1213	hsa_miR_143	-1.3442	516.5192
hsa_miR_27b	1.5093	361.5261			
hsa_miR_29a	1.4927	171.4770			
hsa_miR_452	1.4899	249.7698			
ambi_miR_7084	-1.4868	214.7566			
hsa_miR_523	-1.4806	286.6044			
hsa_miR_31	-1.4742	457.9917			
hsa_let_7g	-1.4714	653.8679			
hsa_miR_122a	-1.4650	608.1613			
rno_miR_297	-1.4500	587.3088			
hsa_miR_19a	1.4485	169.2059			
hsa_let_7c	-1.4417	483.5760			
ambi_miR_7105	-1.4399	33.5882			
hsa_miR_132	1.4372	327.3716			

Clone	Fold Change	Avg. Int.	Clone	Fold Change	Avg. Int.
hsa_miR_380_3p	1.4297	304.7082			
hsa_miR_105	-1.4252	328.6605			
hsa_miR_28	-1.4189	317.3633			
hsa_miR_200b	1.4170	115.5571			
hsa_miR_133a	-1.4122	254.0713			
hsa_miR_30e_3p	-1.4038	572.4405			
mmu_miR_140_AS	-1.4035	179.2206			
hsa_miR_214	1.4016	404.5326			
rno_miR_151_AS	1.4013	312.4533			
hsa_miR_20a	1.4008	148.7541			
hsa_miR_195	-1.3983	319.1480			
hsa_miR_223	-1.3960	415.9830			
hsa_miR_183	-1.3954	603.1783			
hsa_miR_373_AS	1.4631	25.7223			
rno_miR_349	-1.3899	195.2858			
hsa_miR_188	-1.3877	542.2359			
hsa_miR_147	-1.3862	433.9811			
ambi_miR_7058	1.3850	494.5904			
hsa_miR_504	1.3837	439.0520			
hsa_miR_215	-1.3835	323.4792			

Table 4
Microarray analysis during hair cell regeneration. Day 12 vs 0

Clone	Fold Change	Avg. Int.	Clone	Fold change	Avg. Int.
hsa_let_7b	-2.6298	1221.1691	rno_mir_333	-1.5993	893.7625
hsa_mir_520a	2.8071	22.7310	mmu_mir_199b	1.5882	290.6795
hsa_let_7e	-2.4280	1302.4903	hsa_mir_34b	-1.5788	792.3592
hsa_let_7a	-2.2824	1009.1084	hsa_mir_519e	1.5725	303.7151
hsa_let_7c	-2.2726	1103.7925	ambi_mir_7105	1.5725	465.1575
hsa_mir_185	-2.2251	883.8237	hsa_mir_361	-1.5690	903.1490
hsa_mir_21	-2.1342	733.2364	hsa_mir_494	1.5675	633.6184
hsa_mir_374	2.0228	621.8023	hsa_mir_383	-1.6739	19.5692
hsa_mir_325	-1.9874	1078.0184	hsa_mir_142_5p	1.5614	473.3618
hsa_mir_142_3p	-1.9490	911.2375	hsa_mir_147	1.5585	612.7283
ambi_mir_7103	1.9353	292.3189	mmu_mir_192	1.6636	469.8407
mmu_mir_298	-1.9046	954.7137	hsa_let_7f	-1.5535	794.0183
hsa_mir_373_AS	2.0971	25.4489	hsa_mir_135a	-1.5526	693.6723
hsa_mir_136	-1.8915	899.0768	rno_mir_327	-1.5520	726.4895
rno_mir_7_AS	1.8872	478.5270	hsa_mir_496	1.5520	532.1293
mmu_mir_293	-1.8585	816.3131	hsa_mir_485_5p	-1.5515	732.0955
ambi_mir_7081	-1.8509	1007.9606	hsa_mir_379	-1.5440	839.9563
hsa_let_7d	-1.8405	1192.9872	hsa_mir_505	-1.5413	634.0932
hsa_mir_130a	1.8235	448.0113	hsa_mir_27b	1.5404	591.0668
hsa_mir_449	-1.8211	950.3503	hsa_mir_30a_3p	-1.5400	952.4757
hsa_mir_373	-1.8153	623.1538	mmu_mir_329	-1.5399	370.7281
hsa_mir_138	-1.8095	837.4795	hsa_mir_370	-1.5396	699.4478
hsa_mir_23a	-1.7835	109.4549	hsa_mir_374	1.5353	577.6142
hsa_mir_185	-1.7728	807.2191	hsa_mir_518f_AS	1.5328	567.9875
hsa_mir_122a	-1.7689	895.2785	hsa_mir_34b	-1.5324	884.6310
ambi_mir_7058	-1.7548	853.4199	ambi_mir_7097	1.5320	543.8395
mmu_mir_325	-1.7509	821.1835	hsa_mir_30e_5p	-1.5267	794.1920
hsa_let_7g	-1.7462	1061.8068	hsa_mir_128a	1.5242	531.1833
ambi_mir_7055	1.7376	497.0282	hsa_mir_518f	-1.5198	248.5913
hsa_mir_33	-1.7330	1273.6923	hsa_mir_383	-1.5167	10.1160

Clone	Fold Change	Avg. Int.	Clone	Fold change	Avg. Int.
hsa_miR_142_3p	1.7319	382.0059	ambi_miR_7026	-1.5152	402.5555
hsa_miR_296	1.7151	647.2378	hsa_miR_20a	1.5118	310.7852
hsa_miR_452	1.7076	298.4812			
hsa_miR_526b_AS	1.7056	417.6010			
mmu_miR_201	1.7019	551.8309			
hsa_miR_193b	1.6931	431.1058			
hsa_miR_361	-1.6867	838.4150			
hsa_miR_142_3p	-1.6829	933.3106			
hsa_miR_381	1.6790	401.4013			
ambi_miR_7101	1.6704	468.7710			
mmu_miR_207	1.6484	345.1215			
hsa_miR_335	-1.6478	877.1218			
hsa_miR_34a	-1.6438	1059.6713			
hsa_miR_26b	1.6426	493.0280			
mmu_miR_215	1.6348	576.2521			
hsa_miR_202_AS	1.6323	502.1667			
rno_miR_327	-1.6262	619.9649			
hsa_miR_187	-1.6260	928.6982			
hsa_miR_525_AS	-1.6244	567.6541			
hsa_miR_519e_AS	1.6156	599.6974			
mmu_miR_140_AS	1.6112	333.0877			
hsa_miR_425	-1.5995	320.4162			