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MicroRNA expression profiles in head and neck cancer cell lines

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Abstract

Non-coding RNA molecules such as microRNAs (miRNAs) may play an important role in human carcinogenesis. Their expression has been profiled in many human cancers but there are few published studies in head and neck cancer. In this study, the relative expression of 261 mature miRNA genes was determined in nine head and neck cancer cell lines using an oligonucleotide array platform. Thirty-three miRNAs in the array were found to be highly expressed and 22 showed low levels of expression in all cell lines. Notable was the high expression of miR-21 and miR-205. Expression of several miRNAs was validated using Northern blot analysis. Potential targets of validated miRNAs included tumor suppressor genes, kinesin family member 1B isoform alpha (KIF1B), and hypermethylated in cancer 2 (HIC2), and pleomorphic adenoma gene 1 (PLAG1). This study provides the largest genomewide survey of mature miRNA transcripts in head and neck cancer cell lines.

Keywords: MicroRNAs; Head and neck cancer; Genomics; Cancer genetics

MicroRNAs (miRNAs) are defined as small non-coding RNA genes [1] which mediate gene expression at the post-transcriptional level. Hundreds of miRNAs have been identified in various animal genomes. To date there are 471 miRNA genes described in the human genome [2]. Their biological function remains largely unknown. However it is becoming apparent that mammalian miRNAs have the potential to regulate at least 20–30% of all human genes [3]. Evidence is also accumulating indicating that deregulation of specific miRNAs may lead to human diseases such as cancer [4,5].

Numerous studies have highlighted the suspected role of miRNAs in tumorigenesis and established that profiling of these miRNAs represents an informative measure to determine developmental lineage and differentiation state of various human malignancies. In addition, miRNA profiling has shown that hsa-miR-16-1 and has-miR-15a are absent in the majority of chronic lymphocytic leukemias (CLLs) [5] and expression of hsa-miR-216 and hsa-miR-217 has been associated with pancreatic ductal adenocarcinoma [6]. These profiling studies have been further extended to include a number of other leading human cancers such as breast, colon, stomach, and lung [7–10].

One cancer, which has received very little attention in this area of genome profiling, is head and neck cancer [11]. Head and neck cancer is the sixth most common cancer worldwide [12]. The most common type is squamous-cell carcinoma and despite advances in surgical and other treatments survival rates are not improving [13]. While the major risk factors for head and neck cancers are tobacco and alcohol, there is very little information about

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the precise molecular pathways. As such, some of the urgent priorities in this field are the need to identify and elucidate novel gene(s) or pathways that may choreograph this disease.

The major aim of this study was to utilize an oligonucleotide microarray to measure the relative expression of 261 mature miRNAs in head and neck cancer cell lines. These lineages represented distinct cancers originating from the tongue, oropharynx, larynx, and hypopharynx. This study represents the largest survey of head and neck cell cancer lines for miRNA gene expression. In addition, we attempted to seek associations in the literature between these miRNAs and their potential targets for a role in head and neck carcinogenesis.

Materials and methods

Cell lines and tissue culture. Investigations were carried out on nine mammalian cell lines. Details of the cell lines and their corresponding tissue culture techniques are presented in Table 1.

Detection of specific miRNA genes by RNA analysis. RNA was isolated from cell lines using Trizol (Invitrogen™, USA) as recommended by the manufacturer. Twenty micrograms of total RNA was electrophoresed in a 15% TBE–Urea gel using 1× RNA loading buffer (Ambion, USA). Following electrophoresis the RNA was transferred to a nylon membrane (Invitrogen™, USA).

A DNA probe for $tRNA^{val}$ (5′-CTA AGT GTA AGT TGG GTG CTT TGT GTT AAG CTA CAC TC TG-3′) was utilized to quantify the amount of RNA loaded in individual samples. Probes were designed by obtaining miRNA sequences from the online miRbase database (http://microrna.sanger.ac.uk/sequences) and performing a reverse complement calculation.

For radiolabelling short oligonucleotides (<40 base pairs), the end-labelling method was used. Radiolabelled probe was hybridized using ExpressHyb Hybridization Solution (Clontech, USA). The hybridization was performed at 37 °C for 2 h. Membranes were washed with 500 ml of 2× SSC/0.05% SDS for 5–10 min at room temperature and then exposed to a phosphorimaging screen (Amersham Biosciences, USA). The resulting image was scanned on a phosphorimager, Typhoon 8600 Variable Mode Imager (Amersham Biosciences, USA), and analyzed using the ImageQuant software package (Molecular Dynamics, USA).

MicroRNA microarray methods. The relative expression of 261 miRNA genes was examined using a oligonucleotide-array platform [14]. At the time of this study 261 miRNA genes were deposited in miRBase Version 7.0 (Sanger Institute, UK). This collection of miRNA genes was selected as the probe set. Probes (reverse complement sequences) were spotted at a concentration of 50 μM onto the GAPS microarray slide in duplicate. All cell lines were arrayed in duplicate, with each miRNA analyzed in quadruplicate.

Twenty micrograms of mature miRNA was labelled according to published protocols [14]. Slides were pre-hybridized in buffer (3× SSC, 0.1% SDS, and 0.2% bovine serum albumin (BSA) for a minimum of 1 h at 65 °C. After labelling the reaction products were purified using ethanol precipitation. The samples were then dissolved in 6 μL of a Cy-5-labelled reference set to a final dilution of 1:1000. This mixture was then incubated at 65 °C for 3–5 min. Sixty microliters of Church and Gilbert's hybridization solution (400 nM Na_2HPO_4, 5.8% BSA, and 12% formamide) was added and heated at 95 °C for 2 min. The samples were loaded in the reaction chamber and the slide was then placed in a rotating hybridization oven with the rotor set at 5 rpm and hybridized at 37 °C for 2 h.

After hybridization, the slides were washed three times for 5 min in 2× SSC/0.025% SDS, 0.8× SSC and then 0.4× SSC, at rt. This procedure was repeated three times. The slides were then scanned in a GenePix 4000B Scanner from Axon Instruments (Axon Instruments). Scanning wavelengths were set between 600–700 for the red channel (wavelength 635) and 500–700 for the green channel (wavelength 532). Measures of intensity were assigned to each spot on the array using the software GenePix® Pro Version 5.0 (Axon Instruments).

Reference set. The reference set comprised oligonucleotides complementary to each probe on the array slide. The reference set was labelled with Cy5 using a ULYSIS® Nucleic Acid Labeling Kit (Molecular Probes). For bioinformatic, analysis, see Supplementary methods.

Results and discussion

Relative expression of miRNA genes between head and neck cancer cell lines

The profiling data represented nine cell lines from four different anatomical head and neck sites. A reference set (Cy5-labelled) composed of oligonucleotides complementary to each miRNA probe on the array slide was used for the comparative analyses. Each array was co-hybridized with the reference set and sample (Cy3-labelled). In principle, the reference set acts as an internal hybridization control for every probe on the array. Expression of each miRNA was then calculated as a Cy3/Cy5 ratio relative to the reference set. Although each cell line was arrayed in duplicate, the results are presented as single arrayed entities.

Table 1
Summary of human head and neck cancer cell lines used in this study

Cell line	Cell type	Stage	Reference
FaduD	Hypopharyngeal carcinoma	Squamous cell carcinoma of laryngopharynx Grade I1	[30]
$HN6^R$	Base of Tongue primary carcinoma	T3N2bM0	[31]
$HN13^D$	Tongue primary carcinoma	T2N2M0	[31]
$UM\text{-}SCC9^D$	Tongue primary carcinoma	T2N0M0	[32]
$UM\text{-}SCC47^D$	Tongue primary carcinoma	T3N1M0	[32]
$UM\text{-}SCC10A^D$	Larynx primary carcinoma	T3N0M0	[32]
$UM\text{-}SCC11A^D$	Larynx primary carcinoma	T2N2aM0	[32]
$UM\text{-}SCC38^D$	Tonsil primary carcinoma	T2N2aM0	[32]
$UMSCC4^D$	Tonsil primary carcinoma	T3N2aM0	[32]

Media was supplemented with 10% foetal calf serum (JRH Biosciences) and 1% L-glutamine (Invitrogen™). The superscript following the cell line indicates the specific growth medium: ^{R}denotes RPMI (JRH Biosciences™) and ^{D}denotes Dulbecco's modified Eagle's medium (DMEM) (JRH Biosciences™). All UM-SCC cell lines were a generous gift from Dr. Thomas Carey, University of Michigan, USA.

The data were then mined to ask two specific questions: (a) which miRNA genes were expressed in common across all lines? (b) were there any differentially expressed miRNAs genes within this cohort of head and neck cancer cell lines? To address the first question, common miRNA genes were determined using two bioinformatic approaches. First, a one-class *t* test was used with an adjusted Bonferroni correction with hierarchy clustering (HCL). In this instance, we identified 54 commonly expressed miRNA genes. From this set, 31 were highly expressed and 23 showed low levels of expression across the nine cell lines (Fig. 1A).

As a statistical comparison, the same data set was also analyzed using the significance analysis of microarrays (SAM) approach [15]. This analysis was performed with a one-class analysis followed by HCL (Fig. 1B) and yielded 55 commonly expressed miRNAs. Of these, 33 were highly expressed and 22 lowly expressed across all samples. For example, hsa-miR-21 was expressed at a ratio of 3.0 or above across all cell lines. In contrast, hsa-miR-204 expression levels were invariably observed between 0 and −3.0, or below. A comparison between one-class *t* test and SAM indicated that 52 miRNAs were consistently identified across both types of analyses (Supplementary Table 1). hsa-miR-100, hsa-miR-103, and hsa-miR-107 were only retrieved by SAM analyses whereas hsa-miR-204 was identified using the one-class *t* test. The advantage of using SAM approach is the computation of a false discovery rate (FDR). The FDR represents the percentage of genes identified as significant by chance [15]. A low FDR for a significant gene would

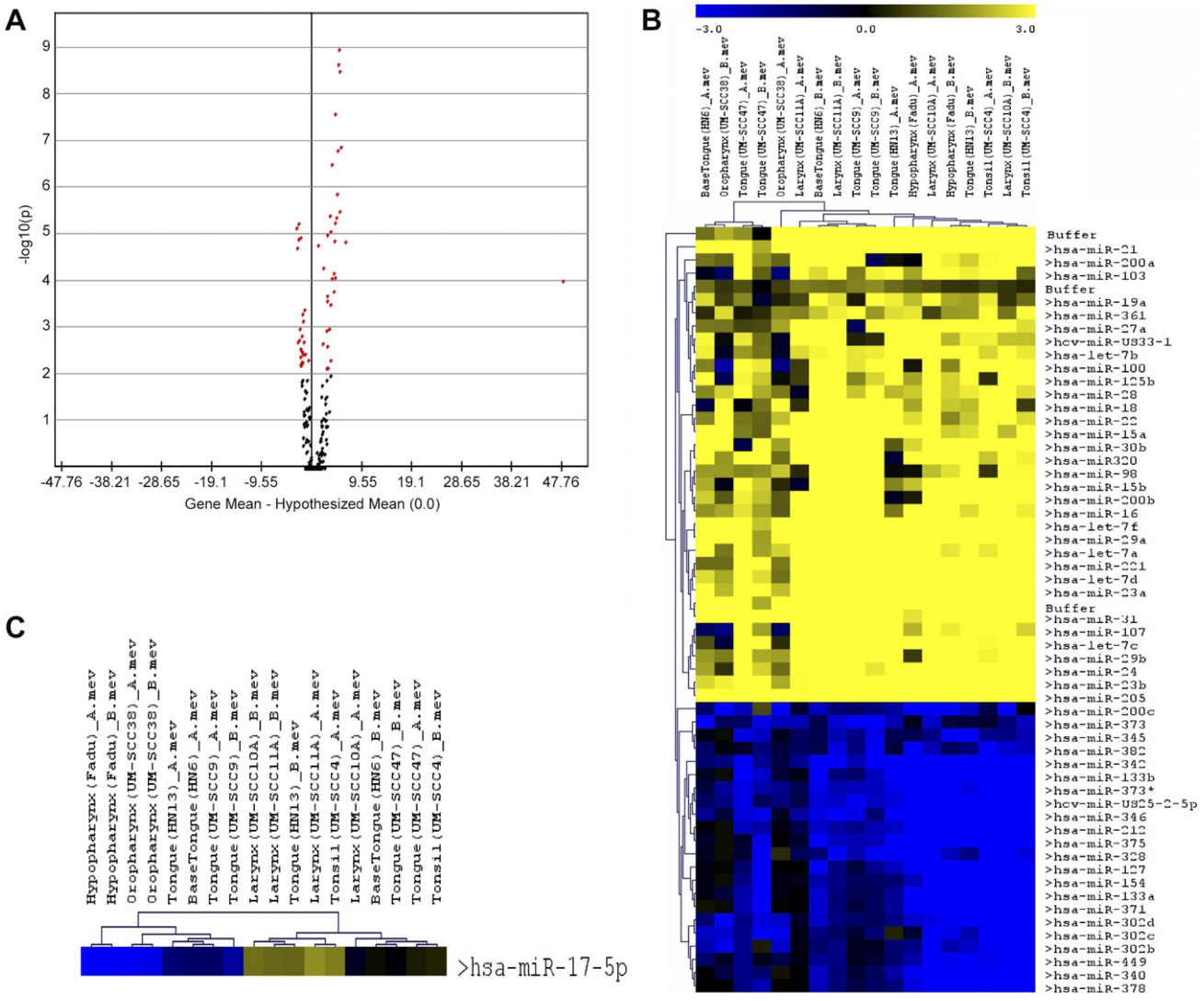

Fig. 1. (A) Volcano plot showing the relative expression of miRNA genes from a one class *t* test. Red dots indicate genes with a positive (highly expressed) or negative (lowly expressed) twofold change. (B) Heat map of positive (yellow) and negative (blue) significant miRNA genes identified using one-class SAM analysis. From this analysis, there were 33 genes highly expressed and 22 genes lowly expressed in all the head and head cancer cell lines. (C) Tissue-specific analysis using two-class SAM approach. The hypopharynx (FaDu) cell line was analysed against all other head and neck cancer cell lines. This analysis yielded one tissue-specific miRNA gene. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

indicate the greater likelihood that it represents a true significant gene, rather than a falsely discovered one. This likelihood would decrease with an increasing FDR. The FDR values in the 90th percentile for the miRNA genes from the one class SAM analysis were <0.01.

Interestingly, hsa-miR-205 which was highly expressed in all of our cell lines was also shown to be overexpressed only in head and neck cancer when compared to other cancer lines from the lung, breast, colorectal, prostate, and pancreas [11]. These findings provide evidence that hsa-miR-205 may be exclusively overexpressed in head and neck tumors.

Due to the unavailability of corresponding normal head and neck lines, we cannot state that these expressed miRNAs reflect a cancerous state. However, hsa-miR-21 which was expressed at high levels in all of our cell lines has been shown to be highly expressed in other malignancies such as glioblastoma [16,17]. Moreover, in a previous study hsa-miR-21 was highly overexpressed in breast tumors compared to matched normal breast tissues [18]. In addition, we are currently profiling tonsil cancers using matched normal and tumor specimens and have found hsa-miR-21 to be overexpressed only in the tumor cohort (unpublished data). Taken together these findings suggest that has-miR-21 may be a cancer-specific miRNA gene.

This study has also identified a group of miRNA genes that were expressed at very low levels across all cell lines. A search of the literature indicated that low level expression of these miRNA genes has not been described to date for any other human cancer or cell lines. Profiling of tumor versus normal tissue in head and neck cancer would be essential to ratify these findings.

Validation of microarray expression by RNA blot analysis

To validate the array data, the expression of seven miRNA genes was determined using Northern blot analysis. These were selected because of their potential to regulate targets which may be involved in cancer. This group consisted of four highly expressed miRNAs (let7-a, miR-16, miR-21, and miR-205) and three showing low level expression in all cell lines (miR-342, miR-346, and miR-373*).

In general, the expression of these seven miRNAs observed in the arrays was consistent with that observed in the Northern blots. All four highly expressed miRNAs were detectable by Northern blot analysis in all nine cell lines although levels were variable (Fig. 2). Due to the higher sensitivity of the arrays, we would expect little or no detection for the lowly expressed miRNAs by Northern blot analysis. As indicated, these lowly expressed miRNAs were undetectable using Northern blots (Fig. 3). For miR-342, only the pre-cursor miRNA was observed in the Northern blots. A positive hybridization control (a DNA oligonucleotide representing the mature miRNA) for both miR-346 and miR-373* was detected on the Northern blot analyses.

Site-specific miRNA gene expression profiles

For the second part of our analysis, we sought to determine differentially expressed miRNA genes between the four anatomically derived cancer cell lines (tongue, oropharynx, larynx, and hypopharynx). This was performed using a two-class unpaired SAM approach which has been previously shown to be successful in identifying tissue or site-specific genes [19]. SAM approach of the three tongue, three oropharyngeal, and two laryngeal cancer cell lines showed an absence of site-specific miRNA gene expression profiles. In comparison, when the same analysis was performed on the hypopharyngeal FaDu cell line, hsa-miR-17-5p was clearly shown to be expressed at lower levels compared to the other cell lines (Fig. 1C). The average Cy3/Cy5 ratio for hsa-miR-17-5p in the hypopharyngeal FaDu line was −4.35. Moreover, the calculated FDR rate in the 90th percentile for this suggested miRNA gene was <0.01.

The lack of differential miRNA expression between the different head and neck cancer lines (e.g., tongue, oropharynx, and larynx) may be a consequence of the limited sample size. We reason that with a larger study, signatures representing the different tumors of the neck and neck will become more apparent. This is supported by published large-scale profiling studies, which have clearly shown that miRNA signatures can succinctly differentiate tumor site. Surprisingly, evidence of a tissue specific miRNA did emerge for the hypopharygeal FaDu cell line. In a very recent study, expression of miR-17-5p was also noted as low in breast cancer cell lines [20]. Furthermore, the low FDR for hsa-mir-17-5p indicates that it most likely represents a genuine low expressed miRNA gene.

Predicted targets of microRNA's genes in head and neck cancer cell lines

MicroRNAs have the potential to regulate diverse target genes including tumor suppressors. However, due to the imperfect binding between the majority of human miRNAs and their targets, individual miRNAs may regulate hundreds of target genes. The identification of targets has been accelerated by the development of several computational algorithms. These databases can be accessed to compile potential targets for all miRNAs genes. On the notion that these potential targets are purely speculative we sought to explore if these head and neck miRNAs could be involved in the regulation of cancer-related genes.

Three online databases miRander [21], and PicTar [22], TargetScan 3.0 [3], were utilized for obtaining predicted gene targets. We restricted this search to the seven miRNAs genes which were validated from the Northern blot data. As expected these miRNA genes could potentially regulate several hundred targets (Supplementary Table 2). The number of predicted gene targets for miR-21 was similar across the three databases with an average of 146 gene targets. In comparison, the number of predicted target genes for miR-346 varied greatly from 73

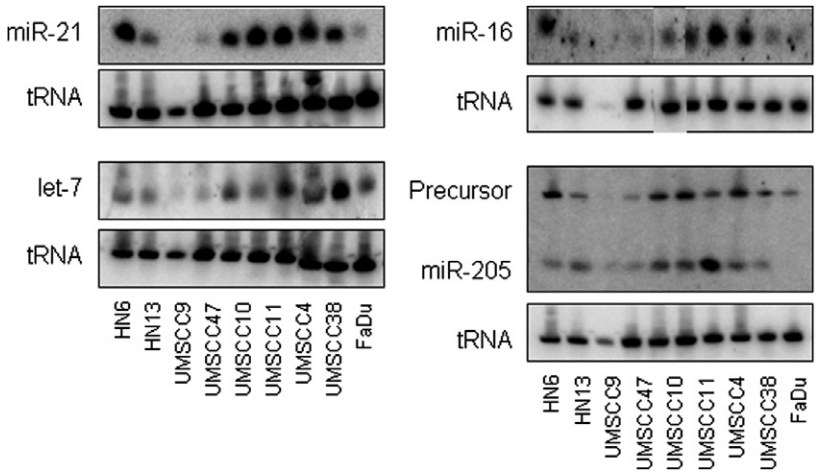

Fig. 2. RNA blot analysis for miR-21. let-7, miR-16, and miR-205 in head and neck cancer cell lines. The top panel represents mature miRNA expression and the bottom panel indicates tRNA expression.

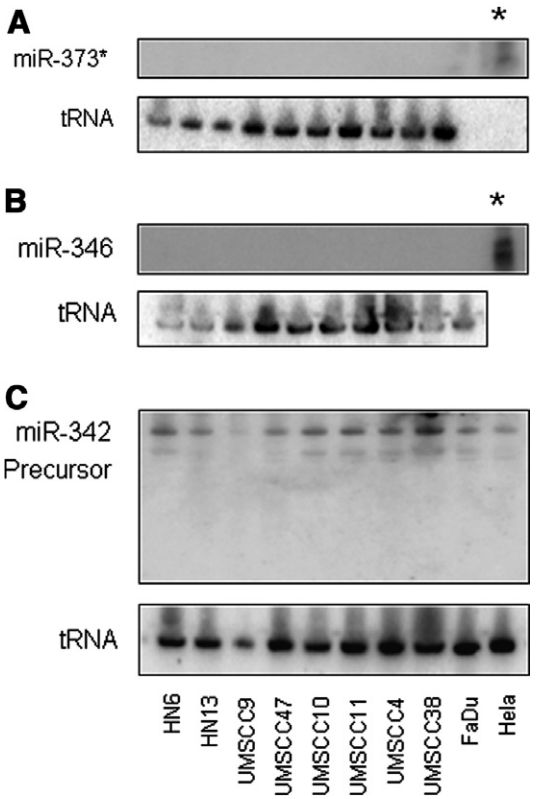

Fig. 3. Northern blots for lowly expressed miRNA genes. Top panel depicts mature miRNAs whilst bottom panel represents tRNA expression. *, Positive control. (A) miR-373*, (B) miR-346, and (C) miR-342. Sample lanes are as shown in (C).

(using PicTar and TargetScan 3.0), to 1775 (using miRanda). miR-16 and miR-205 showed the highest number of predicted genes. The next step was to determine the top three target genes for each individual miRNA. Recently a study indicated that PicTar had the highest success rate in target gene prediction [23]. Supplementary Table 3 provides a summary showing the top three predicted target genes, according to their PicTar score. Of the 21 target genes identified by PicTar, 13 were also identified by the database TargetScan 3.0.

From the top three predicted target genes for each of the seven selected miRNAs, six have been noted to potentially play a role in carcinogenesis: pleomorphic adenoma gene 1 (PLAG-1) [24], hypermethylated in cancer 2 (HIC2) [25], activity-dependent neuroprotector (ADNP) [26], kinesin family member 1B isoform alpha (KIF1B) [27], high mobility group AT-hook 2 (HMGA2) [28], and B-cell CLL/lymphoma 6 (zinc finger protein 51) BCL6 [29]. Potential miRNA binding sites in the 3′ UTR of these target genes are shown in Supplementary Table 3. Notably, the let-7a family has up to seven different binding sites on the HMGA2 gene.

It is important to remember that these are predicted targets and experimental validation of these targets is necessary to determine a bona fide regulatory relationship. Nonetheless, it is exciting to speculate that these miRNAs may represent the first non-coding genes to be implicated in tumorigenesis of the head and neck region. One of the strongest links to support the notion of a role for specific miRNA genes in head and neck cancer is the regulation of HMGA2 by miR-98. This miRNA has been shown to be upregulated in selected head and neck cancer lines grown under hypoxic conditions [28]. In addition miR-98 was also identified with high expression levels across all of the cell lines in our study.

In summary, the focus of this study was to investigate the relative expression of mature miRNA genes in head and neck cancer cell lines. This is one of the first studies of this nature in head and neck cancer lines. Previous studies of smaller numbers of cell lines have profiled the expression of precursor miRNAs [11] or examined mature miRNA expression under specific growth conditions [28]. The exact function of these miRNA genes remains to be determined but we believe this study provides a basis for further investigation of their function in head and neck cancer.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.03.201.

References

[1] M. Lagos-Quintana, R. Rauhut, W. Lendeckel, T. Tuschl, Identification of novel genes coding for small expressed RNAs, Science 294 (2001) 853–858.
[2] S. Griffiths-Jones, The microRNA Registry, Nucleic Acids Res. 32 (2004) D109–D111.
[3] B.P. Lewis, C.B. Burge, D.P. Bartel, Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets, Cell 120 (2005) 15–20.
[4] N. Yanaihara, N. Caplen, E. Bowman, M. Seike, K. Kumamoto, M. Yi, R.M. Stephens, A. Okamoto, J. Yokota, T. Tanaka, G.A. Calin, C.G. Liu, C.M. Croce, C.C. Harris, Unique microRNA molecular profiles in lung cancer diagnosis and prognosis, Cancer Cell 9 (2006) 189–198.
[5] G.A. Calin, M. Ferracin, A. Cimmino, G. Di Leva, M. Shimizu, S.E. Wojcik, M.V. Iorio, R. Visone, N.I. Sever, M. Fabbri, R. Iuliano, T. Palumbo, F. Pichiorri, C. Roldo, R. Garzon, C. Sevignani, L. Rassenti, H. Alder, S. Volinia, C.G. Liu, T.J. Kipps, M. Negrini, C.M. Croce, A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia, N. Engl. J. Med. 353 (2005) 1793–1801.
[6] A.E. Szafranska, T.S. Davison, J. John, T. Cannon, B. Sipos, A. Maghnouj, E. Labourier, S.A. Hahn, MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma, Oncogene (2007).
[7] J. Lu, G. Getz, E.A. Miska, E. Alvarez-Saavedra, J. Lamb, D. Peck, A. Sweet-Cordero, B.L. Ebert, R.H. Mak, A.A. Ferrando, J.R. Downing, T. Jacks, H.R. Horvitz, T.R. Golub, MicroRNA expression profiles classify human cancers, Nature 435 (2005) 834–838.
[8] S. Volinia, G.A. Calin, C.G. Liu, S. Ambs, A. Cimmino, F. Petrocca, R. Visone, M. Iorio, C. Roldo, M. Ferracin, R.L. Prueitt, N. Yanaihara, G. Lanza, A. Scarpa, A. Vecchione, M. Negrini, C.C. Harris, C.M. Croce, A microRNA expression signature of human solid tumors defines cancer gene targets, Proc. Natl. Acad. Sci. USA 103 (2006) 2257–2261.
[9] M.V. Iorio, M. Ferracin, C.G. Liu, A. Veronese, R. Spizzo, S. Sabbioni, E. Magri, M. Pedriali, M. Fabbri, M. Campiglio, S. Menard, J.P. Palazzo, A. Rosenberg, P. Musiani, S. Volinia, I. Nenci, G.A. Calin, P. Querzoli, M. Negrini, C.M. Croce, MicroRNA gene expression deregulation in human breast cancer, Cancer Res. 65 (2005) 7065–7070.
[10] H. He, K. Jazdzewski, W. Li, S. Liyanarachchi, R. Nagy, S. Volinia, G.A. Calin, C.G. Liu, K. Franssila, S. Suster, R.T. Kloos, C.M. Croce, A. de la Chapelle, The role of microRNA genes in papillary thyroid carcinoma, Proc. Natl. Acad. Sci. USA 102 (2005) 19075–19080.
[11] J. Jiang, E.J. Lee, Y. Gusev, T.D. Schmittgen, Real-time expression profiling of microRNA precursors in human cancer cell lines, Nucleic Acids Res. 33 (2005) 5394–5403.
[12] D.M. Parkin, Global cancer statistics in the year 2000, Lancet Oncol. 2 (2001) 533–543.
[13] N. Tran, B.R. Rose, C.J. O'Brien, Role of human papillomavirus in the etiology of head and neck cancer, Head Neck 29 (2007) 64–70.
[14] J.M. Thomson, J. Parker, C.M. Perou, S.M. Hammond, A custom microarray platform for analysis of microRNA gene expression, Nat. Methods 1 (2004) 47–53.
[15] V.G. Tusher, R. Tibshirani, G. Chu, Significance analysis of microarrays applied to the ionizing radiation response, Proc. Natl. Acad. Sci. USA 98 (2001) 5116–5121.
[16] J.A. Chan, A.M. Krichevsky, K.S. Kosik, MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells, Cancer Res. 65 (2005) 6029–6033.
[17] S. Diederichs, D.A. Haber, Sequence variations of microRNAs in human cancer: alterations in predicted secondary structure do not affect processing, Cancer Res. 66 (2006) 6097–6104.
[18] M.L. Si, S. Zhu, H. Wu, Z. Lu, F. Wu, Y.Y. Mo, miR-21-mediated tumor growth, Oncogene (2006) 1–5.
[19] R. Shyamsundar, Y.H. Kim, J.P. Higgins, K. Montgomery, M. Jorden, A. Sethuraman, M. van de Rijn, D. Botstein, P.O. Brown, J.R. Pollack, A DNA microarray survey of gene expression in normal human tissues, Genome Biol. 6 (2005) R22.
[20] A. Hossain, M.T. Kuo, G.F. Saunders, Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA, Mol. Cell. Biol. 26 (2006) 8191–8201.
[21] B. John, A.J. Enright, A. Aravin, T. Tuschl, C. Sander, D.S. Marks, Human MicroRNA targets, PLoS Biol. 2 (2004) e363.
[22] A. Krek, D. Grun, M.N. Poy, R. Wolf, L. Rosenberg, E.J. Epstein, P. MacMenamin, I. da Piedade, K.C. Gunsalus, M. Stoffel, N. Rajewsky, Combinatorial microRNA target predictions, Nat. Genet. 37 (2005) 495–500.
[23] D. Grun, Y.L. Wang, D. Langenberger, K.C. Gunsalus, N. Rajewsky, MicroRNA target predictions across seven *Drosophila* species and comparison to mammalian targets, PLoS Comput. Biol. 1 (2005) e13.
[24] J. Declercq, F. van Dyck, C.V. Braem, I.C. van Valckenborgh, M. Voz, M. Wassef, L. Schoonjans, B. van Damme, L. Fiette, W.J. van de Ven, Salivary gland tumors in transgenic mice with targeted PLAG1 proto-oncogene overexpression, Cancer Res. 65 (2005) 4544–4553.
[25] S. Deltour, S. Pinte, C. Guerardel, D. Leprince, Characterization of HRG22, a human homologue of the putative tumor suppressor gene HIC1, Biochem. Biophys. Res. Commun. 287 (2001) 427–434.
[26] R. Zamostiano, A. Pinhasov, E. Gelber, R.A. Steingart, E. Seroussi, E. Giladi, M. Bassan, Y. Wollman, H.J. Eyre, J.C. Mulley, D.E. Brenneman, I. Gozes, Cloning and characterization of the human activity-dependent neuroprotective protein, J. Biol. Chem. 276 (2001) 708–714.
[27] H. Caren, K. Ejeskar, S. Fransson, L. Hesson, F. Latif, R.M. Sjoberg, C. Krona, T. Martinsson, A cluster of genes located in 1p36 are down-regulated in neuroblastomas with poor prognosis, but not due to CpG island methylation, Mol. Cancer 4 (2005) 10.
[28] C. Hebert, K. Norris, M.A. Scheper, N. Nikitakis, J.J. Sauk, High mobility group A2 is a target for miRNA-98 in head and neck squamous cell carcinoma, Mol. Cancer 6 (2007) 5.
[29] B.W. Baron, G. Nucifora, N. McCabe, R. Espinosa 3rd, M.M. Le Beau, T.W. McKeithan, Identification of the gene associated with the recurring chromosomal translocations t(3;14)(q27;q32) and t(3;22)(q27;q11) in B-cell lymphomas, Proc. Natl. Acad. Sci. USA 90 (1993) 5262–5266.
[30] S.R. Rangan, A new human cell line (FaDu) from a hypopharyngeal carcinoma, Cancer 29 (1972) 117–121.
[31] M. Cardinali, H. Pietraszkiewicz, J.F. Ensley, K.C. Robbins, Tyrosine phosphorylation as a marker for aberrantly regulated growth-promoting pathways in cell lines derived from head and neck malignancies, Int. J. Cancer 61 (1995) 98–103.
[32] E. Virolainen, R. Vanharanta, T.E. Carey, Steroid hormone receptors in human squamous carcinoma cell lines, Int. J. Cancer 33 (1984) 19–25.