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臨床組-從業

頭頸癌中女性相較於男性之miRNAs 變化之研究

miRNAs Changes of Female Compared to Male in Head and Neck Cancer

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ABSTRACT

In Taiwan, oral cancer (OC) causes more than 2000 deaths each year and is, at present, the fourth leading cause of cancer death in men. Especially in south Taiwan, the incidence of oral cancer is high and further increasing and is associated with many deaths. Head and neck /oral squamous cell carcinomas (HNOSCC) represent the most frequent of all oral neoplasms, more than 90% of all oral neoplasms are estimated to be HNSOSCC, and it has a very high recurrence rate. The 5-year survival rate of oral cancer is 60–80% when detected during its early stages; however, the early identification of recurrence or second primary tumors remains a challenge. Therefore, an early detection method for the diagnosis and prognosis of HNOSCC to increase long-term patient survival is urgently needed and to further improve patient outcomes, better biomarkers and therapeutic strategies also are necessary.

Accumulating evidence suggest that microRNAs (miRNAs) play important roles in human cancers. They are pivotal regulators of diverse cellular processes including proliferation, differentiation, apoptosis, survival, motility, and morphogenesis. Whether microRNA expression patterns can be used for early diagnosis and prognosis of head and neck cancer is an important issue. However, most studies of miRNAs focus on male patients of HNOSCC, a few of study was investigated with female miRNAs in this issue. In clinic, we can find an upward trend in female with HNOSCC. Accordingly, it is interested us to focus on female case and hypothesize that female and male may be different with onmiRNAs expression of HNOSCC. For further exploring this novel issue, we studied this proposal suing female HNOSCC patients and mainly in a population-based case-control study. We showed the results that fold change of miRNA-7 (mir-7) and miRNA-21 (mir-21) exhibited significantly different expression level between male and female in specimen HNOSCCs.

INTRODUCTION

Head and neck cancer is the sixth most common cancer in the world. The most common type is head and neck/oral squamous cell carcinoma (HN/OSCC). Despite in surgical and other treatments, the survival rate remains at 50-60 %, often due to the cancer recurrence (Forastiere et al. 2001). HN/OSCC has a poor clinical prognosis and usually results in local or regional recurrence. In Taiwan, oral cancer (OC) including oral cavity, oropharynx and hypopharynx melanoma, causes more than 2000 deaths each year and is, at present, the fourth leading cause of cancer death in men reported by Taiwan Cancer Registry. Therefore, identification of explicate standardized molecular markers that assist in determining HNC prognosis is necessary.

MicroRNAs (miRNAs) investigation is a promising field of study that may lead to the discovery of novel biomarkers for prognosis and novel therapeutic targets. The study of miRNAs and its role in the molecular mechanism of human cancer diseases has been an area of prevailing research (Chang et al. 2008). miRNAs are defined as small non-coding RNA genes that mediate gene expression at the posttranscriptional level by degrading or repressing target messenger RNAs (mRNA) by base pairing to partially complementary sites, predominantly in the 3′ untranslated region of mRNA (Lagos-Quintana et al. 2001). Hundreds of miRNAs have been identified in various animal genomes. Accumulating evidence also shows that deregulation of specific miRNAs may lead to human diseases such as cancer, including HNOC (Liu et al. 2009).

Recent advances in miRNA expression profiling have led to a better understanding of cancer pathogenesis, inflammation, and metastasis. To date, numerous studies have highlighted the role of miRNAs in tumorigenesis and have established that profiling of these miRNAs represents an informative measure to determine the developmental lineage, differentiation state and clinic prognosis of HNOC (Liu et al. 2009). Moreover, a total of 6843 patients, in the majority of male HNOSCC patients, across 50 studies were included in the systematic review revealed that miRNAs have potential clinical value as prognostic biomarker, especial to miRNA-7, miR-21, 125b and 34c-5p, showing great potential as prognostic molecular markers (Chellan et al. 2019).

However, most studies of miRNAs focus on male patients of HNOSCC, a few of study was investigated with female miRNAs in HNOSCC. In clinic, we can find an upward trend in female with HN/OSCC. Therefore, identification of miRNAs profiling may strategize this disease in female is necessary. The main aim of this study is to exploit miRNAs array profiling technologies and HNOSCC specimens, along with detailed clinic-pathology features, to globally analyze miRNAs expression signatures in female HNOSCC patients. Finally, we expect to confirm our hypothesis both miRNAs' the expression in male and female are differently, and to identify potential different biomarkers for prognosis and predicting the metastasis, with as therapeutic target in HN/SOCC.

MATERIALS AND METHODS

Tissue sampling Institutional Review Board (IRB) at E-Da Hospital already had approved the project. The fresh head and neck/oral cancer tissue and non-tumor specimens of patients will be applied from E-Da Hospital Tissue Bank. Details of clinical characteristics, pathologic results, TMN staging status, treatment modalities and treatment responses will be obtained.

miRNA analysis for profiling miRNA expression pattern in patient Agilent Human miRNA microarray experiments were be carried out to analyze the miRNA expression profile in both OSCCs and normal tissues. Moreover, we selected several miRNAs interested us for validation by RT-qPCR analysis and attempt to predict potential prognostic marker of male and female patients by specific miRNAs.

Statistical analysis Student T-test was used for comparing of variables. Suing a $P < 0.05$ to determine the significant level. All analysis were performed by SPSS 19.0 and Primer5 for Windows and P value of less than 0.05 was considered statistically significant in two-tail.

RESULTS

miRNA array analysis for profiling miRNA expression pattern in male patients

The preliminary results of using microRNA microarray analysis for profiling miRNA expression from 40 HNOSCC male patients indicated that 31 miRNAs overexpressed and 51 miRNAs down-expressed ($p = 0.05$) with the fold change >2, respectively. The change fold (log2 scale) was calculated with the tumor vs. no-tumor ratio of miRNAs expression level in each patient's pair (see the Table). The data was be indicated miRNAs overexpression or miRNAs down-expression with the fold change >2, respectively. The change fold (log2 scale) was calculated with the tumor vs. no-tumor ratio of miRNAs expression level in each patient's pair.

Table: miRNA differentially expressed in tumor to matched nontumor tissue samples of OSCCs

	Microarray (n=40 pairs)			RT-qPCR (training set, n=20 pairs)		
miRNA	Fold changes[1]	*p* value	Concordance[2]	Fold changes[1]	*p* value	Concordance[2]
Overexpressed						
has-miR-7	1.75	2.84E-13	33, 82.5%	3.43	1.60E-05	20, 100%
has-miR-21*	2.43	2.97E-15	36, 90%	2.72	8.51E-06	17, 85%
has-miR-31	1.32	4.91E-13	24, 60%	3.79	2.06E-03	18, 90%
has-miR-31*	2.06	1.35E-09	30, 75%	4.08	4.40E-03	17, 85%
has-miR-424	1.32	1.84E-15	25, 62.5%	2.89	5.64E-06	18, 90%
has-miR-424*	1.73	4.28E-08	29, 72.5%	2.40	9.54E-06	18, 90%
has-miR-455-5p	1.38	1.77E-12	26, 65%	1.87	2.34E-04	12, 60%
has-miR-455-3p	1.32	9.81E-13	26, 65%	1.55	2.81E-04	12, 60%
has-miR-503	2.28	2.43E-12	36, 90%	2.92	1.67E-06	18, 90%
Downexpressed						
has-let-7c	-1.55	3.05E-15	33, 82.5%	-2.60	5.78E-05	18, 90%
has-miR-1	-2.53	6.02E-08	30, 75%	-4.51	2.42E-03	17, 85%
has-miR-29c*	-1.29	1.09E-11	29, 72.5%	-1.83	2.28E-04	13, 65%
has-miR-30a	-1.55	1.50E-15	32, 80%	-1.34	4.34E-04	19, 95%
has-miR-30a*	-2.00	3.19E-10	33, 82.5%	-2.69	1.84E-05	12, 60%
has-miR-99a	-1.26	2.73E-18	28, 70%	-2.24	4.08E-04	15, 75%
has-miR-133a	-1.53	1.33E-08	28, 70%	-5.36	1.93E-04	18, 90%
has-miR-139-5p	-1.67	3.64E-14	33, 82.5%	-2.48	1.27E-06	18, 90%
has-miR-154	-1.70	1.76E-12	300, 75%	-2.56	1.26E-04	15, 75%
has-miR-299-5p	-2.08	5.05E-08	32, 80%	-4.33	7.95E-05	19, 95%
has-miR-337-3p	-1.82	1.70E-10	33, 82.5%	-3.08	3.83E-05	17, 85%
has-miR-375	-3.76	5.19E-08	34, 85%	-6.17	6.09E-03	18, 90%
has-miR-376c	-1.43	2.11E-11	28, 70%	-2.86	4.73E-05	16, 80%
has-miR-378*	-1.72	4.08E-08	28, 70%	-2.80	3.99E-05	17, 85%
has-miR-411	-2.14	2.34E-12	33, 82.5%	-2.77	1.52E-04	17, 85%
has-miR-487b	-1.98	2.19E-11	35, 87.5%	-3.12	2.29E-05	18, 90%
has-miR-495	-1.49	2.58E-07	29, 72.5%	-2.88	3.25E-04	17, 85%
has-miR-499-5p	-2.00	6.41E-08	30, 75%	-4.92	1.55E-03	18, 90%

[1] Mean fold changes (log2 scale) of miRNA from 40 tissues pairs and 20 training set pairs (t-test).
[2] The concordance is represented the numbers of the frequencies in 40 tissues pairs and 20 training set pairs in which the ratio of the miRNA expression is over 2-fold changed.

RT-qPCR analysis for validation miRNA expression pattern in female and male patients

We predict there were different expression patterns of miRNA profiling between male and female patients. Accordingly, we selected several miRNAs (mir-7 and mir-21) interested us for validation by RT-qPCR analysis and attempt to predict potential prognostic marker of male and female patients by specific miRNAs. The results showed that fold change of miRNA-7 (mir-7) ($p < 0.0001$) and miRNA-21 (mir-21) ($p = 0.005$) exhibited significantly different expression level between male and female in specimen OSCCs (see the Figure).

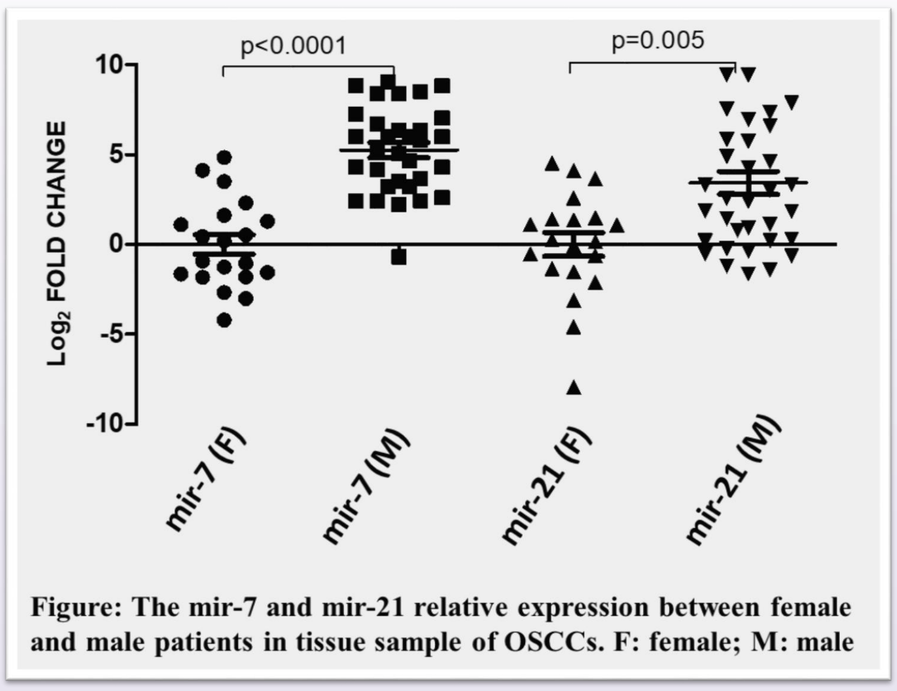

Figure: The mir-7 and mir-21 relative expression between female and male patients in tissue sample of OSCCs. F: female; M: male

CONCLUSIONS

We confirmed our hypothesis that miRNAs' expression in male and female are different in mir-7 and mir-21. Moreover, we expect to identify other potential miRNAs or predicting prognosis and metastasis biomarkers for different therapeutic strategies in female or male HNSOCCs.

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