Gene 671 (2018) 96–102

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Long non-coding RNA biomarker for human laryngeal squamous cell carcinoma prognosis

Jingjing Chen[a,1], Zhisen Shen[a,1], Hongxia Deng[a], Wei Zhou[b], Qi Liao[c], Ying Mu[d,*]

[a] Department of Otorhinolaryngology (Head and Neck Surgery), Ningbo City Medical Treatment Center Lihuili Hospital, Ningbo 315000, Zhejiang, China
[b] Departmcnt of Colorectal Surgery, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China, 310016
[c] Department of Preventative Medicine, Zhejiang Provincial Key Laboratory of Pathological and Physiological Technology, Medical School of Ningbo University, Ningbo 315211, Zhejiang, China
[d] EagleImmune, Inc., 11361 Bishops Gate Ln, Laurel, MD 20723, USA

ARTICLE INFO

Keywords:
Laryngeal squamous cell carcinoma
lncRNA AC008440.10
Biomarker
Lymph node metastasis
Prognosi

ABSTRACT

Long non-coding RNAs (lncRNA) were discovered in tumors. The regulation of lncRNA in human laryngeal squamous cell carcinoma (LSCC) remains incomplete. Uncovering the potential of lncRNA to stratify the prognosis of LSCC and streamline the vast amount of clinical information will affect medical interventions. The surgical resected LSCC tissues, adjacent non-cancerous tissues (ANCT) and lymph node metastatic tissue (LNM) were collected from 76 patients for lncRNA AC008440.10 expression assay. The stages of LSCC and LNM were classified accordingly. We integrated the epigenetic information with enhanced CT imaging and pathological evaluations to predict the patients' survival by comprehensive statistical algorithms using equal weighting. Significant downregulation of lncRNA AC008440.10 was detected in LSCC tumor and metastatic lymph node in advanced stage of patient samples compared with those in early stage. The pattern of differentially expressed AC008440.10 displayed a clear trend that significantly related to tumor progression. The downregulation of lncRNA AC008440.10 correlates with increasing risk of metastasis, poor prognosis and patient survival. The potential for lncRNA AC008440.10 to be developed as a novel biomarker for stratification of the prognosis was especially promising when clinic parameters were hybridized with equal weight, and using a panel of complementary parameters yielded a more powerful predictability of LSCC prognosis than any single parameter individually.

1. Introduction

Human laryngocarcinoma is the 21st most common cancer in the world; it is the 14th leading cause of cancer death among men and the 23rd leading cause of cancer death among women (Ferlay et al., 2015; Mirisola et al., 2011). Approximately 95% of laryngocarcinoma have histologic patterns corresponding to squamous cell carcinoma (Almadori et al., 2005). Laryngeal squamous cell carcinoma (LSCC) is an aggressive malignancy disease consequently resulting into a poor clinical prognosis. Nearly 160,000 new cases of LSCC are diagnosed and 83,000 deaths occur worldwide every year (Ramroth et al., 2011). The regional recurrence rate of patients in tumor stage I and stage II ranges from 5 to 25% and 15 to 50%, respectively (Devlin and Langer, 2007; Lefebvre et al., 2004). The heterogeneity of LSCC with the intrinsic subtypes and epigenetic signatures during tumor progression makes developing tumor molecular markers for patient outcome and treatment response especially important. Biomarkers are commonly used in the clinic as diagnostic and monitoring tools that offer distinct and obvious advantages for improving clinic outcome (Biomarkers Definitions Working, 2001).

Two types of RNA that are potential cancer biomarkers are non-coding RNA (ncRNA) and mircoRNA (miRNA). At least four times more ncRNA exist than coding RNAs (Lander et al., 2001), or the number of protein-coding genes in the human genome (Kapranov et al., 2007). Conventionally, the ncRNA family is divided into two groups by molecular size: small ncRNA (the length is shorter than 200 nt) and long-non-coding RNA (lncRNA; the length is > 200 nt) (Prensner and Chinnaiyan, 2011). Considered to be genomic “junk,” lncRNA have

Abbreviations: lncRNA, Long non-coding RNAs; LSCC, laryngeal squamous cell carcinoma; ANCT, adjacent non-cancerous tissues; ncRNA, non-coding RNA; miRNA, mircoRNA; WHO, World Health Organization; AJCC, the American Joint Committee on Cancer; TNM, tumor-node-metastasis; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LSD, Least-Significant Difference; SNK, Student–Newman–Keuls; AUC, the area under the curve

* Corresponding author at: 11361 Bishops Gate Ln, Laurel, MD 20723, USA.
E-mail address: yingm6@gmail.com (Y. Mu).

[1] As *co-first author*, Jingjing Chen and Zhisen Shen contributed equally to this work.

https://doi.org/10.1016/j.gene.2018.05.046
Received 22 March 2018; Accepted 13 May 2018

recently gained widespread attention as critical regulators of coding RNA and alternative splicing, and their dysregulation has been associated with tumorigenesis, tumor progression, and metastasis. LncRNA represent a vast source of largely unstudied potential molecular drivers of human cancer, emerging only recently as a new class of promising cancer biomarkers and therapeutic agents (Yarmishyn and Kurochkin, 2015). Unlike protein-coding RNAs, lncRNA show complexity in modulating tumor occurrence. Over the last decade, lncRNA have been extensively investigated in tumorigenesis (Y Wang et al., 2015a). Wang et al. found that the levels of CCAT2 were significantly higher in gastric cancer tissues than in those of corresponding noncancerous tissues and patients with higher expression had poorer prognosis (CY Wang et al., 2015b). Recently, a new type of prognostic marker, termed lncRNA-LET, was identified in cervical cancer; lower expression of LET indicated the tumor was progressing and that the patient was in relapse (Jiang et al., 2015). The other type of RNA for a potential cancer biomarker is miRNA (Park et al., 2015; Shen et al., 2012).

Because our understanding of the relationship between molecular alterations and pathogenesis is vague, our need for more extensive tumor molecular markers with clinical multi-parameters is evident. How can we simplify the vast amount of multi-biological changes that occur over tumor progression in order to achieve an optimal clinical outcome? We hypothesized that combining lncRNA information with clinical signatures such as X-ray computed tomography (CT) image could effectively determine the status of the tumor and assess the outcome. In addition, currently, no reports exist concerning lncRNA as biomarkers for the stratification of the stages or the prognosis of LSCC. Indeed, by envisioning the potential of the tumor molecular markers, we carefully programed a series of studies to discover clinically relevant biomarkers by conducting a RNA microarray study to broadly explore the gene expression profile in the tumor tissues and in their adjacent non-cancerous tissues (ANCT) (Shen et al., 2014). To follow up on our previous study, an lncRNA named AC008440.10 as a known lncRNA (ENST00000422045), a 555 nt transcript, located on the forward strand of chromosome 19:53,864,763–53,866,140 was selected for further evaluation by performing quantitative real-time PCR (qPCR) and multiple comparisons across tissues using expression of lncRNA AC008440.10, CT imaging, morphology, and patients survival data accordingly in this confirmatory study. Notably, we found a clear relationship between lncRNA AC008440.10 and the stages of LSCC, lymph node metastatic tissue (LNM), and survival.

2. Materials and methods

2.1. Samples preparation and clinical information

All patients were diagnosed as having primary LSCC and classified according to the latest World Health Organization (WHO) criteria (Edge and Compton, 2010). At the point of surgery, the age patients were presented as the oldest, mean, and youngest as 86, 61, and 39, respectively. Seventy-six patient samples of LSCC and ANCT were paired, and 28 patients with LNM samples of lymph node were unpaired that were obtained while patients received the surgical resection of LSCC from January 2009 through May 2014. The tissues were divided into two groups according to intensive CT image, group 1 displayed neck lymph node swelling (n = 28) and group 2 did not (n = 48). All samples were immediately frozen in liquid nitrogen and then stored at −80 °C until assayed. During the surgical dissection, we remove the tumor from possibly visible boundary between tumor and normal tissue, then, take a quick cryostat slide for a microscopic observation by pathologist to determine whether the cutoff reached a normal tissue. For the negative control, a normal tissue was collected in a distance about 5 cm away from the tumor boundary with using clean tools. The CT based image classification is intended to guide whether the patient needs an extended drain lymph nodes removal. If the CT image showed negatively, the procedure was omitted. Only CT image showed positive lymph node metastasis/enlarged were removed and a pathological examination and lncRNA expression of those lymph nodes were performed. All tissue samples were pathologically evaluated by two specialized pathologists according to the American Joint Committee on Cancer 2002 (AJCC 2012) tumor-node-metastasis (TNM) staging criteria (Edge and Compton, 2010). Clinical information of patients with LSCC is summarized in Table 1.

Table 1
Characteristics of the 76 LSCC patients.

Characteristic	Total
Age, mean ± SEM (rang), y	61 ± 1.124 (39–86)
Male	73
Female	3
Smoker (no.) mean ± SEM (range), py	(57) 101.8 ± 7.78 (30 − 300)
Nonsmoker (no.)	(19)
LNM CT imaging	
Positive	28
Negative	48
TNM criteria	
T1	21
T2	22
T3	15
T4	18
N0	48
N1	13
N2	15
N3	0
Follow-up term, mean ± SEM (range), m	39.26 ± 2.1 (5.8–68.8)
Survival	59
Death	8
Irrelevant death	1
Discontinued	8

y: year.
m: month.
no.: number.
py: pack year.
All patients that gave written informed consent were notified that their medical records were authorized only for research purposes. This study was approved by the Human Research Ethics Committee of Ningbo University.

2.2. RNA extraction

The frozen laryngeal tumors and ANCT were minced and homogenized (IKA, Staufen, Germany) and the total RNA was extracted using TRIZol or TRIZol LS (Invitrogen, Karlsruhe, Germany). The concentrations and quality of the RNA were semi-quantitated by a UV spectrophotometer (Agilent Technologies, Hercules, CA, USA). The purity of the RNAs was examined by absorbance of 260/280 nm ratio, the RNAs ranging from 1.8 to 2.0 were reversely transcribed to cDNAs according to the manufacturer's protocol (Promega, Madison, WI, USA) (Sun et al., 2013).

2.3. Quantitative polymerase chain reaction

The RNA samples were amplified by qPCR using GoTaq qPCR master mix (Promega) on a Mx3005P QPCR System (Stratagene, La Jolla, CA, USA). All samples were performed in triplicate. The annealing temperature was set at 58 °C. The sequences of primers for AC008440.10 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are listed in Table 2. The expression of AC008440.10 was normalized by the ΔCt method using GAPDH as housekeeper gene. All data used in this context were constrained by normalization. The qPCR conditions were incubated at 95 °C for 10 min followed by 45 cycles of 95 °C for 15 s, 56 °C for 30 s, and 72 °C for 30 s.