**RESEARCH**                                                                                      **Open Access**

CrossMark

# Low let-7d and high miR-205 expression levels positively influence HNSCC patient outcome

Tomasz Kolenda[1,2,3*], Kacper Guglas[1,3], Anna Teresiak[1], Renata Bliźniak[1] and Katarzyna Lamperska[1*]

## Abstract

**Introduction:** Head and neck squamous carcinoma (HNSCC) is one of the most invasive types of cancer with high mortality. A previous study has indicated that low levels of let-7d and miR-205 in HNSCC patients are correlated with poor survival. Let-7d and miR-205 are tumor suppressors and regulators of epithelial-to-mesenchymal transition (EMT). However, it is unclear if let-7d and miR-205 together influence cancer cells.

**Aim:** To determine if let-7d and miR-205 expression levels influence HNSCC patient outcome.

**Methods:** The TCGA expression data for let-7d, miR-205 and their targets as well as clinical data were downloaded from cBioPortal and starBase v2.0 for 307 patients. The expression levels of let-7d and miR-205 were verified according to clinicopathological parameters. The let-7d and miR-205 high- and low-expression groups as well as disease-free survival (DFS), overall survival (OS) and expression levels of genes related to EMT, cancer stem cells, metastasis, cell cycle, drug response and irradiation response were investigated.

**Results:** Let-7d and miR-205 were frequently upregulated in HNSCC compared to normal samples, and ROC analysis showed high discrimination ability for let-7d and miR-205 (area 0.7369 and 0.7739, respectively; $p < 0.0001$). Differences between expression levels of let-7d or miR-205 and grade, angiolymphatic invasion, perineural invasion and alcohol consumption were indicated. No differences were observed in N-stage, tumor localization, gender or patient age. Patients with lower let-7d levels and higher miR-205 levels had significantly better OS ($p = 0.0325$) than patients with higher let-7d levels and lower miR-205 levels. In the low let-7d level and high miR-205 level group, a lower percentage of more advanced cancers was observed. The analysis of genes related to EMT, cancer stem cells, metastasis, cell cycle, drug response and irradiation response revealed a distinct phenotype of analyzed groups.

**Conclusions:** The present findings indicated that let-7d down-regulation and miR-205 overexpression create a unique cell phenotype with different behavior compared to cells with upregulated let-7d and down-regulated miR-205. Thus, let-7d and miR-205 are good candidates for new HNSCC biomarkers.

**Keywords:** Head and neck cancer, miRNA, Gene expression, Biomarker

* Correspondence: kolenda.tomek@gmail.com; kasialam@o2.pl
[1]Laboratory of Cancer Genetics, Greater Poland Cancer Centre, 15th Garbary Street, room 5025, 61-866 Poznan, Poland
Full list of author information is available at the end of the article

## Introduction

Head and neck squamous cell carcinoma (HNSCC) is characterized by high mortality and difficult to treat type of cancer. The main treatment of HNSCC involves surgical resection, radiotherapy and chemotherapy [1, 2]. Many reports have shown a close connection between HNSCC and miRNAs [3]. miRNAs are RNAs that are 22 nucleotides long and function as posttranscriptional regulators of specific mRNA by targeting the 3' UTR of mRNA, resulting in reduced expression of the encoded proteins. These small RNAs regulate many biological processes, such as cell cycle, apoptosis, EMT, cancer-initiating cells, metastasis, drug response and irradiation response. The implication of miRNAs in HNSCC development and progression is well documented. miRNAs may function as tumor suppressors and/or oncogenes [3, 4], and they may be used as potential biomarkers in oncology [5, 6]. Childs et al. proposed that low expression levels of let-7d and miR-205 are prognostic markers of HNSCC [7], but other reports have not confirmed this finding.

Expression of lethal-7d (let-7d) is dysregulated in many types of cancer, such as HNSCC, breast cancer, kidney cancer, retinoblastoma, pancreatic cancer and prostate cancer [4, 8]. Let-7d plays a crucial role in cancer development, progression and metastasis, and it acts as a tumor suppressor miRNA through regulating the expression of many oncogenes [4]. However, recent studies have indicated that let-7d also acts as an oncogene [9, 10]. Let-7d regulates the response to irradiation and drug exposure through changes in cell phenotype via the EMT process or by changes in multidrug resistant genes [11–13].

MiR-205 is altered in many cancers, including breast cancer, melanoma, renal cancer, glioblastoma, lung cancer and HNSCC [6, 7, 14, 15]. MiR-205 may function as a tumor suppressor, and it has been described as an epithelial marker [16, 17] and a modulator of the EMT process [15]. In contrast, miR-205 functions as an oncogene in breast cancer, cervical cancer and nasopharyngeal carcinoma [18, 19].

The present study analyzed the expression of let-7d and miR-205 in HNSCC patients based on TCGA data. The aim of the present study was to investigate the influence of let-7d and miR-205 expression levels on HNSCC patient outcome.

## Methods

### TCGA data

TCGA expression data of let-7d, miR-205 and selected genes (cell cycle, apoptosis, EMT, cancer-initiating cells, metastasis, irradiation response and drug response) as well as clinical data were downloaded from cBioPortal (head and neck squamous cell carcinoma, TCGA, Provisional, 530 sample data set) and starBase v2.0 for 307 patients and presented as miRNAseq RPM (Reads Per Million) and mRNA expression z-Scores (RNA Seq V2 RSEM; z = 2, RNA-Seq by Expectation Maximization) [20, 21]. All data is available online with common access.

### Data analysis

The expression levels of let-7d and miR-205 were analyzed in all HNSCC sample localizations depending on the following clinicopathological parameters: age (< 60 vs. > 60), gender (women vs. men), T-stage (T1 + T2 vs. T3 + T4), N-stage (N0 vs. N1 + N2 + N3), cancer grade (G1 + G2 vs. G3 + G4), cancer stage (I + II vs. III + IV), HPV p16 marker (negative vs. positive), perineural invasion (negative vs. positive), angiolymphatic invasion (negative vs. positive), disease surgical margin status (negative vs. positive) and lymphoid neck dissection status (negative vs. positive). The group of 307 patients was divided into high and low expression subgroups for let-7d and/or miR-205. The following groups were created based on cut-off values for miRNA expression of healthy samples: i) high let-7d + low miR-205 (N = 24 cases); ii) high let-7d + high miR-205 (N = 217 cases); iii) low let-7d + low miR-205 (N = 12 cases) and iv) low let-7d + high miR-205 (N = 54 cases). Disease free-survival (DFS) and overall survival (OS) were analyzed in the four subgroups. The selected clinicopathological parameters (cancer grade, cancer stage, T-stage, perineural invasion, angiolymphatic invasion and disease surgical margin status) were compared between the low let-7d level and high miR-205 level group and the high let-7d level and low miR-205 level group. The expression levels of selected genes related to cell cycle, apoptosis, EMT, cancer-initiating cells, metastasis, drug response and irradiation response were analyzed. Let-7d and miR-205 predicted targets were verified using the miRDB database [22] and target genes were compared between patients with the low let-7d level and high miR-205 level group and the high let-7d level and low miR-205 level group.

### Statistical analysis

All statistical analyses were performed by Graphpad Prism 5 (GraphPad Software San Diego, CA, USA) using the Shapiro-Wilk normality test, T-test, Mann–Whitney U test or one-way ANOVA with Dunn's multiple comparison test. For DSF and OS analyses, the log-rank (Mantel-Cox) and Gehan-Breslow-Wilcoxon tests were used. In all analyses, a $p$-value < 0.05 was considered as significant. The heat map and clustering were generated using MORPHEUS, an online versatile matrix visualization and analysis software tool (https://software.broadinstitute.org/morpheus/).
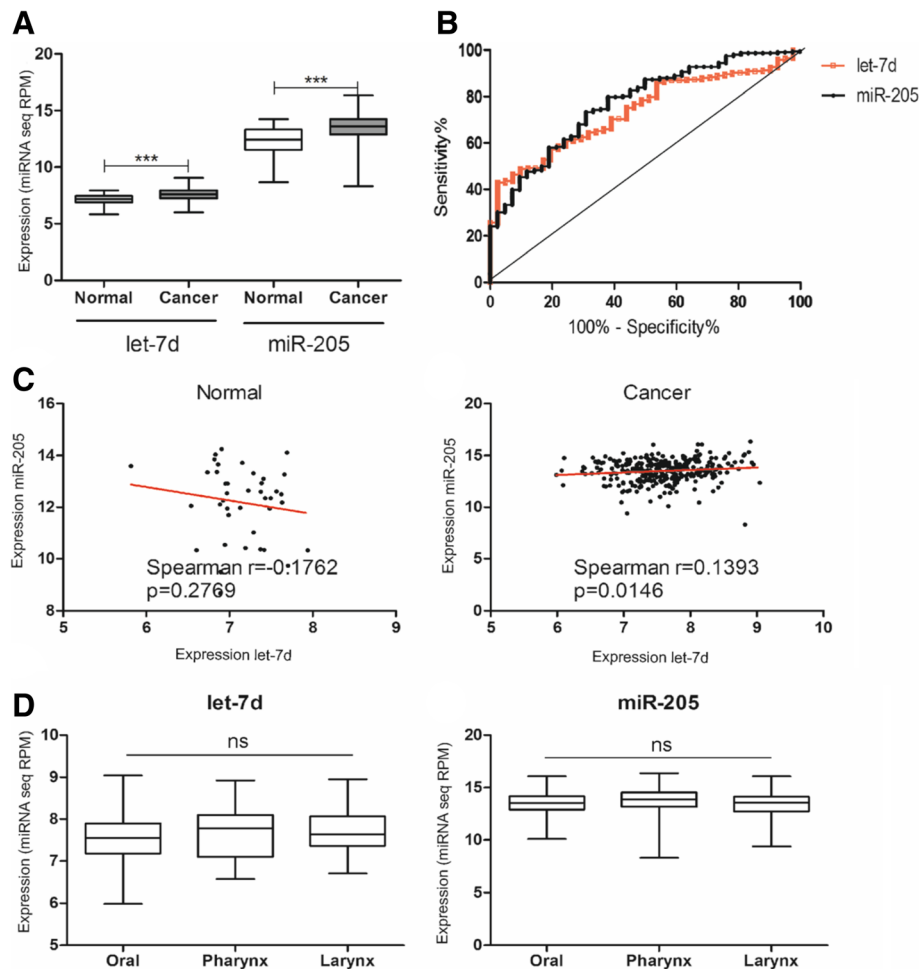
**Fig. 1** Characteristics of let-7d and miR-205 expression in normal and HNSCC tissues. **a**) Expression levels of let-7d and miR-205 in normal and cancer tissues; **b**) ROC analysis of let-7d and miR-205 expression; **c**) Spearman correlation of let-7d and miR-205 in normal and cancer tissues; **d**) Expression of let-7d and miR-205 depending on the tumor localization site. Mean of miRNAseq RPM expression with SEM; T-test or One-way ANOVA with Dunn's Multiple Comparison Test; $p < 0.05$ was considered significant; ns – no significant, *** $p < 0.001$

## Results

### Let-7d and miR-205 are frequently upregulated in HNSCC

Expression levels of let-7d and miR-205 in normal ($N = 41$ and $N = 42$ cases, respectively) and HNSCC tissues ($N = 307$ cases) were analyzed based on TCGA data (starBase v2.0). Both let-7d and miR-205 were frequently upregulated in HNSCC compared to normal samples (let-7d: $7.594 \pm 0.03263$ vs. $7.157 \pm 0.06295$, $p < 0.0001$; and miR-205: $13.50 \pm 0.06195$ vs. $12.21 \pm 0.2130$, $p < 0.0001$) (Fig. 1a). ROC analysis showed high discrimination ability of let-7d (AUC = 0.7369) and miR-205 (AUC = 0.7739) (both $p < 0.0001$) to discriminate cancer and healthy tissues (Fig. 1b).

The correlations between let-7d and miR-205 were also investigated. For normal samples, Spearman correlation analysis indicated no association between the let-7d and miR-205 miRNAs (R = − 0.1762, $p = 0.2769$).

For cancer tissues, however, a positive correlation between let-7d and miR-205 expression levels was observed (R = 0.1393, $p = 0.0146$) (Fig. 1c).

The expression levels of let-7d and miR-205 were examined based on the oral ($N = 193$), pharynx ($N = 39$) and larynx ($N = 75$ cases) tumor localizations. No significant differences between localization and expression of both let-7d ($p = 0.1305$) and miR-205 ($p = 0.1126$) were identified (Fig. 1d).

### Expression level of let-7d and miR-205 reflecting clinicopathological parameters of HNSCC patients

The expression levels of let-7d and miR-205 were analyzed depending on clinicopathological context. Significant differences between expression levels of let-7d or miR-205 were observed with regard to grade ($p = 0.0002$ for let-7d; $p = 0.5185$ for miR-205), angiolymphatic

invasions ($p = 0.0024$ for let-7d; $p = 0.008$ for miR-205), perineural invasions ($p = 0.5565$ for let-7d; $p = 0.0165$ for miR-205), alcohol consumption ($p = 0.0227$ for let-7d; $p = 0.5678$ for miR-205) and neck lymph node dissection ($p = 0.1731$ for let-7d; $p = 0.0091$ for miR-205). There were no statistically significant differences in the expression levels with regard to gender ($p = 0.2445$ for let-7d; $p = 0.6210$ for miR-205), patient age ($p = 0.3846$ for let-7d; $p = 0.8371$ for miR-205), N-stage ($p = 0.0729$ for let-7d; $p = 0.3485$ for miR-205), disease surgical margin status ($p = 0.5931$ for let-7d; $p = 0.1907$ for miR-205), HPV presence ($p = 0.2600$ for let-7d; $p = 0.1429$ for miR-205), cancer stage ($p = 0.6672$ for let-7d; $p = 0.3658$ for miR-205) and T-stage ($p = 0.4987$ for let-7d; $p = 0.5219$ for miR-205). All data is summarized in Table 1.

### Expression levels of let-7d and miR-205 influence patient survival

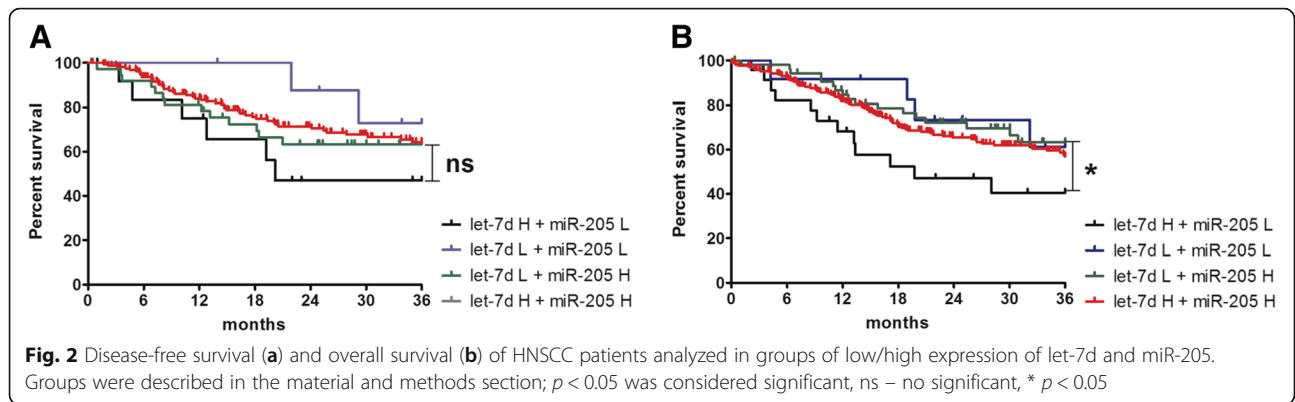The disease-free survival (DFS) and overall survival (OS) of HNSCC patients defined after 36 months of observation were analyzed in groups of different expression levels of let-7d and miR-205. There were no statistically significant differences between groups of patients with specified low and/or high expression levels of let-7d and miR-205. However, patients with low let-7d levels and high miR-205 levels had significantly better OS than patients with opposite phenotype - high let-7d levels and low miR-205 levels ($p = 0.0325$). These results are shown in Fig. 2a and b. Due to differences in survival between these groups, it was checked whether they differ in terms of other clinical features.

### Patients with low let-7d and high miR-205 expression levels have better prognosis

The clinicopathological parameters were analyzed in the low let-7d and high miR-205 expression level group and the high let-7d and low miR-205 expression level group. The low let-7d and high miR-205 expression level group had lower percentages of more advanced cancers compared to the high let-7d and

**Table 1** Expression levels of let-7d and miR-205 depending on the clinicopathological parameters in HNSCC patients; T-test, $p < 0.05$ was considered significant

| Parameter | Group | Cases analyzed | let-7d | | miR-205 | |
|---|---|---|---|---|---|---|
| | | | Mean ± SEM | *p*-val | Mean ± SEM | *p*-val |
| Age | < 61.3 | 159 | 7.621 ± 0.04545 | 0.3846 | 13.49 ± 0.08659 | 0.8371 |
| | > 61.3 | 147 | 7.564 ± 0.04723 | | 13.51 ± 0.08951 | |
| Gender | Female | 95 | 7.537 ± 0.06120 | 0.2445 | 13.59 ± 0.09850 | 0.621 |
| | Male | 221 | 7.619 ± 0.03845 | | 13.46 ± 0.07811 | |
| Alcohol | Yes | 206 | 7.650 ± 0.03906 | 0.0227 | 13.5 ± 0.07550 | 0.5678 |
| | No | 97 | 7.491 ± 0.05784 | | 13.5 ± 0.1092 | |
| Grade | G1 + G2 | 219 | 7.515 ± 0.03841 | 0.0002 | 13.48 ± 0.06774 | 0.5185 |
| | G3 + G4 | 80 | 7.796 ± 0.06118 | | 13.57 ± 0.1461 | |
| Cancer Stage | I + II | 74 | 7.554 ± 0.06325 | 0.6672 | 13.52 ± 0.1002 | 0.3658 |
| | III + IV | 197 | 7.587 ± 0.04059 | | 13.39 ± 0.08370 | |
| T Stage | T1 + T2 | 118 | 7.555 ± 0.05406 | 0.4987 | 13.42 ± 0.08618 | 0.5219 |
| | T3 + T4 | 154 | 7.602 ± 0.04398 | | 13.44 ± 0.09825 | |
| N Stage | N0 | 154 | 7.531 ± 0.04499 | 0.0729 | 13.55 ± 0.07323 | 0.3485 |
| | N1 + N2 + N3 | 140 | 7.651 ± 0.04992 | | 13.41 ± 0.1054 | |
| Perineural Invasion | Yes | 105 | 7.555 ± 0.05409 | 0.5565 | 13.27 ± 0.1050 | 0.0165 |
| | No | 110 | 7.601 ± 0.05588 | | 13.62 ± 0.1045 | |
| Angiolymphatic Invasion | Yes | 67 | 7.762 ± 0.05671 | 0.0024 | 13.15 ± 0.1663 | 0.008 |
| | No | 139 | 7.506 ± 0.05074 | | 13.59 ± 0.07981 | |
| Disease Surgical Margin Status | Negative | 210 | 7.550 ± 0.03765 | 0.5931 | 13.45 ± 0.07263 | 0.1907 |
| | Positive | 30 | 7.607 ± 0.09610 | | 13.23 ± 0.1742 | |
| Lymph Node Neck Dissection Indicator | Yes | 246 | 7.575 ± 0.03530 | 0.1731 | 13.43 ± 0.07065 | 0.0091 |
| | No | 59 | 7.688 ± 0.08177 | | 13.79 ± 0.1249 | |
| HPV p16 status | Negative | 16 | 7.730 ± 0.1301 | 0.26 | 13.63 ± 0.4052 | 0.1429 |
| | Positive | 48 | 7.552 ± 0.07919 | | 13.32 ± 0.1816 | |

**Fig. 2** Disease-free survival (**a**) and overall survival (**b**) of HNSCC patients analyzed in groups of low/high expression of let-7d and miR-205. Groups were described in the material and methods section; $p < 0.05$ was considered significant, ns – no significant, * $p < 0.05$
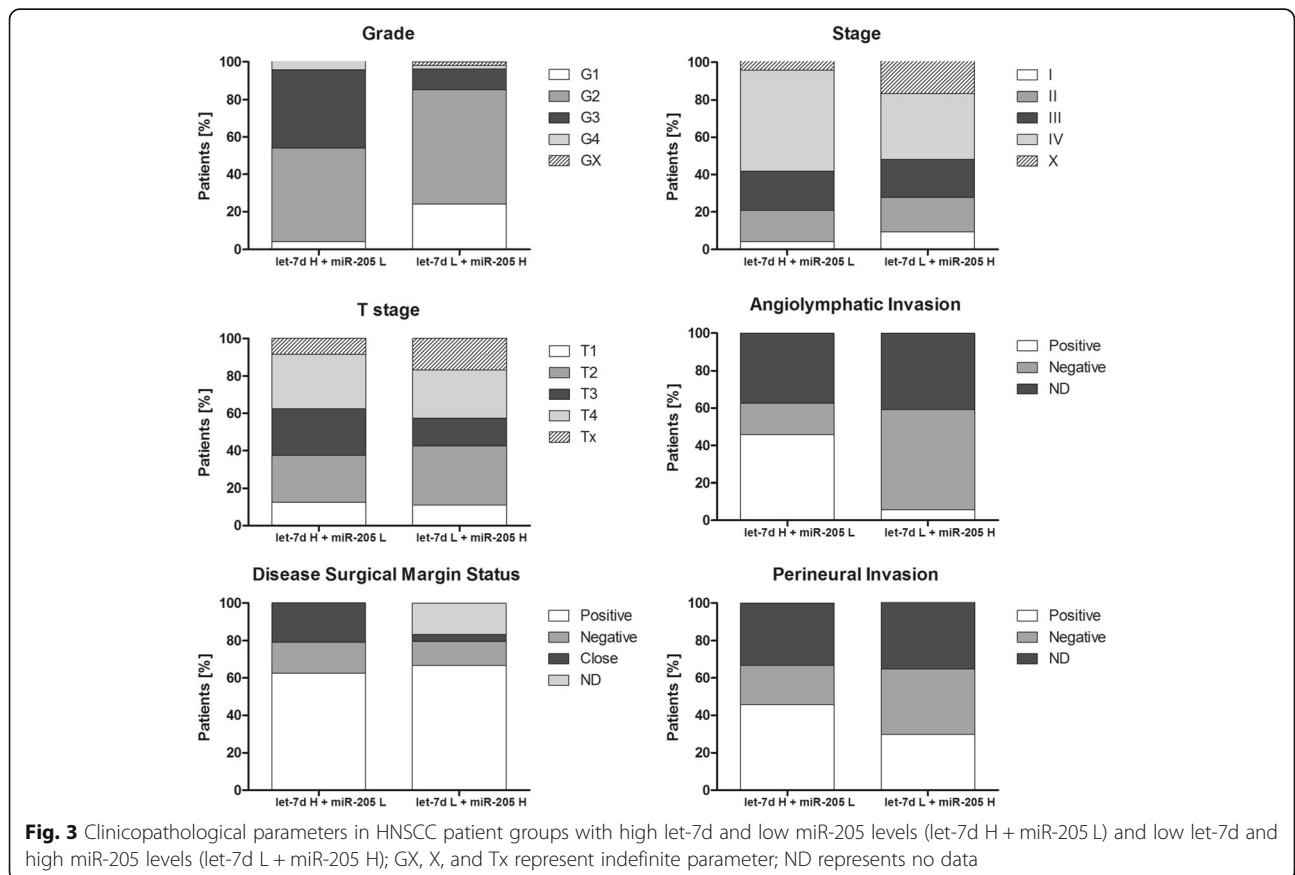
low miR-205 expression level group as indicated by the following parameters: cancer grade (G1 + G2: 85.18% vs. 54.17%; G3: 11.11% vs. 41.67%, respectively), cancer stage (I + II: 27.78% vs. 20.84%; III + IV: 37.56% vs. 75.00%, respectively), T-stage (T1 + T2: 42.59% vs. 37.50%; T3 + T4: 42.60% vs. 54.17%, respectively), angiolymphatic invasion (positive: 5.56% vs. 45.83%; negative: 53.70% vs. 16.67%, respectively), disease status of surgical margin (positive: 66.67% vs.

62.50%; negative: 12.96% vs. 16.67%, respectively), and perineural invasion (positive: 29.63% vs. 45.83%; negative: 35.19% vs. 20.83%, respectively). Graphical presentation of the results is shown in Fig. 3.

## Patients with low let-7d and high miR-205 expression level have a distinct tumor phenotype

For the high let-7d and low miR-205 expression level group (let-7d H + miR-205 L) and the low let-7d and



**Fig. 3** Clinicopathological parameters in HNSCC patient groups with high let-7d and low miR-205 levels (let-7d H + miR-205 L) and low let-7d and high miR-205 levels (let-7d L + miR-205 H); GX, X, and Tx represent indefinite parameter; ND represents no data

high miR-205 expression level group (let-7d L + miR-205 H), the expression levels of genes related to metastasis, EMT, cancer-initiating cells, cell cycle, apoptosis, irradiation response and drug response were examined and compared between these two groups. A distinct expression pattern of these genes was observed for each group. The let-7d L + miR-205 H group showed the following statistically significant ($p < 0.05$) changes compared to the let-7d H + miR-205 L group: 29 down-regulated and 6 upregulated genes related to metastasis; 31 down-regulated and upregulated 8 genes related to EMT; 28 down-regulated and 5 upregulated genes related to cancer-initiating cells; 17 down-regulated and 4 upregulated genes related to cell cycle; 24 down-regulated and 10 upregulated genes related to apoptosis; 32 down-regulated and 5 upregulated genes related to irradiation response; and 44 down-regulated and 9 upregulated genes related to drug response (Figs. 4 and 5).

Next, target genes for let-7d and miR-205 were selected from the studied processes using the miRDB database. Twelve targets, including 11 upregulated targets, were identified for let-7d (patients with high let-7d vs. low let-7d) in the set of genes related to metastasis, EMT, cancer-initiating cells, cell cycle, apoptosis, irradiation response and drug response. The following targets were upregulated: DAPK1 ($0.7075 \pm 0.2553$ vs. $-0.1161 \pm 0.1453$; $p = 0.0039$) and FAS ($0.1111 \pm 0.1606$ vs. $-0.4269 \pm 0.1002$; $p = 0.0046$), which are related to negative regulation of apoptosis; FASLG ($0.2874 \pm 0.2214$ vs. $-0.3418 \pm 0.06343$; $p = 0.0006$), which is involved in positive apoptosis regulation; COL3A1 ($0.6484 \pm 0.2238$ vs. $-0.04903 \pm 0.08634$; $p = 0.0007$), COL5A2 ($0.5338 \pm 0.1996$ vs. $0.02531 \pm 0.09580$; $p = 0.0112$), COL1A2 ($0.6935 \pm 0.2510$ vs. $0.006604 \pm 0.1066$; $p = 0.0039$) and SNAI3 ($0.5260 \pm 0.1833$ vs. $-0.2556 \pm 0.1024$; $p = 0.0002$), which are related to EMT; transmembrane receptor ITGB3 ($1.027 \pm 0.2215$ vs. $-0.1574 \pm 0.1167$; $p < 0.0001$) and CCL7 cytokine, which are related to cell growth and proliferation ($0.05701 \pm 0.05865$ vs. $-0.1297 \pm 0.01475$; $p < 0.0001$); and OGG1 ($-0.3112 \pm 0.2123$ vs. $-0.8249 \pm 0.08819$; $p = 0.0096$) and SMC1A ($0.4036 \pm 0.2117$ vs. $-0.2798 \pm 0.1203$; $p = 0.0038$), which are related to DNA damage and repair. Only one let-7d target was down-regulated, namely CDKN1A ($-0.6176 \pm 0.1373$ vs. $0.3994 \pm 0.1293$; $p < 0.0001$), which is related to cell cycle arrest (Fig. 6a). The following three molecular targets were identified for miR-205 (low miR-205 vs. high miR-205 expression): LTA ($0.1778 \pm 0.1251$ vs. $-0.2701 \pm 0.07117$; $p = 0.0014$), which is a positive regulator of apoptosis; ZEB1 ($1.294 \pm 0.2672$ vs. $-0.05386 \pm 0.1134$; $p < 0.0001$), which is a regulator of cell migration, metastasis, growth and proliferation; and CDH11 ($1.092 \pm 0.2816$ vs. $-0.1691 \pm 0.09423$; $p < 0.0001$), which is related to cell adhesion (Fig. 6b).
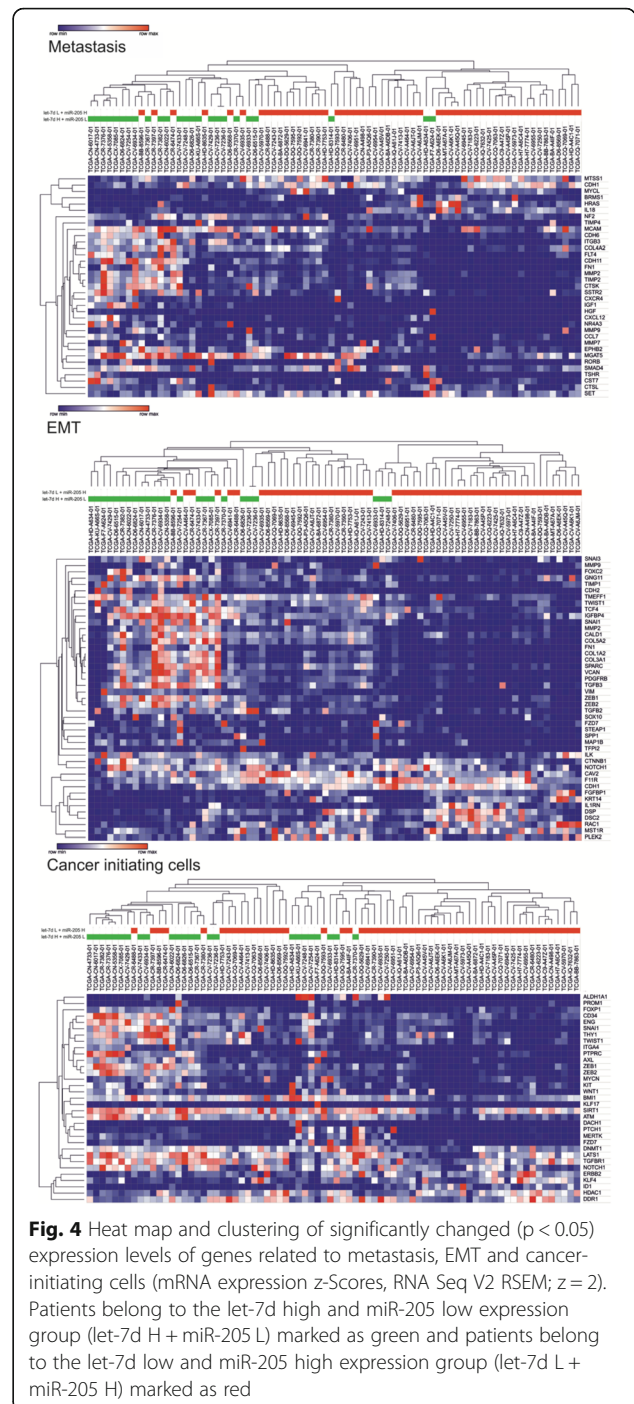


**Fig. 4** Heat map and clustering of significantly changed (p < 0.05) expression levels of genes related to metastasis, EMT and cancer-initiating cells (mRNA expression z-Scores, RNA Seq V2 RSEM; z = 2). Patients belong to the let-7d high and miR-205 low expression group (let-7d H + miR-205 L) marked as green and patients belong to the let-7d low and miR-205 high expression group (let-7d L + miR-205 H) marked as red

## Discussion

Head and neck squamous cell carcinoma (HNSCC) is a group of poor outcome cancers due to late diagnosis, aggressiveness and high capability of metastasis to lymph nodes and distant sites [1, 2, 23]. HNSCC treatment is based on surgery, chemotherapy and radiotherapy, but these treatment strategies are still not personalized and not matched to molecular features of every patient's cancer [2, 5]. Moreover, HNSCCs contain a small
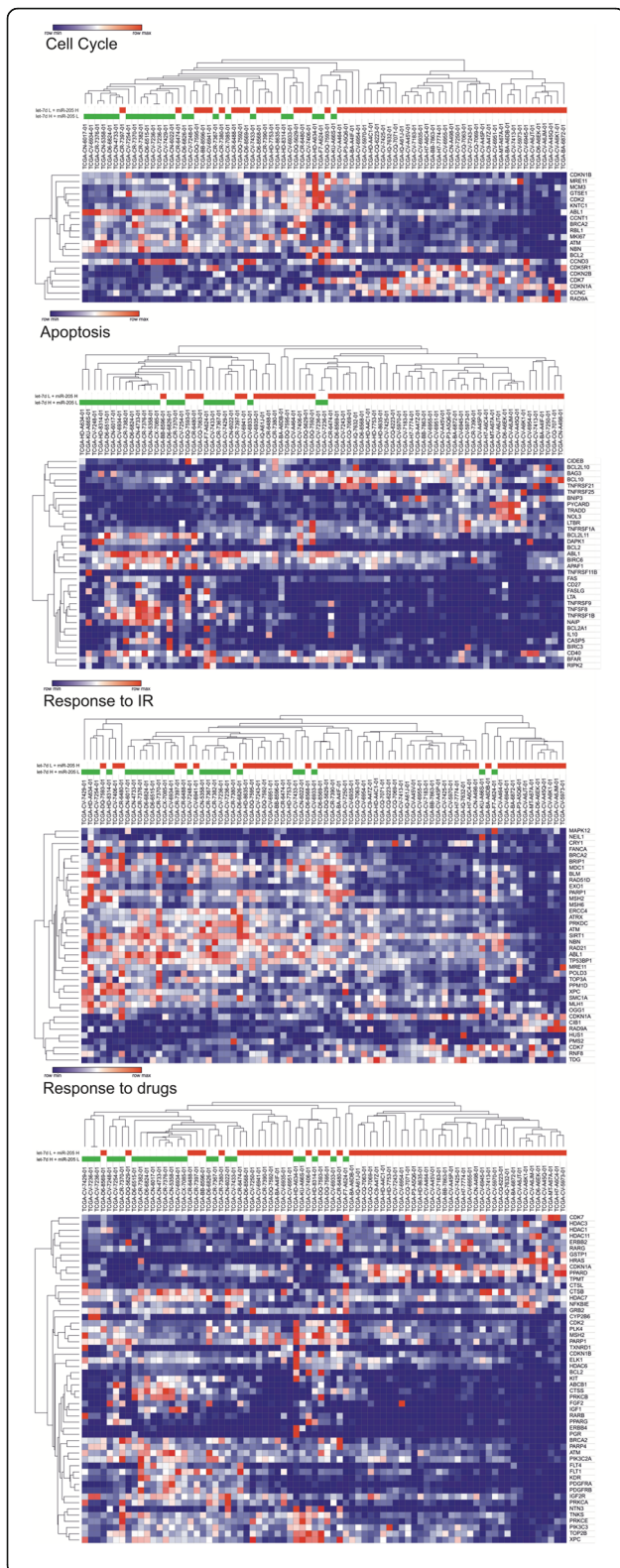
**Fig. 5** Heat map and clustering of significantly changed ($p < 0.05$) expression levels of genes related to cell cycle, apoptosis, irradiation response and drug response (mRNA expression z-Scores, RNA Seq V2 RSEM; z = 2). Patients belong to the let-7d high and miR-205 low expression group (let-7d H + miR-205 L) marked as green and patients belong to the let-7d low and miR-205 high expression group (let-7d L + miR-205 H) marked as red

population of tumorigenic and therapy-resistant cells called cancer-initiating cells (CICs). The epithelial-to-mesenchymal (EMT) process is one of the factors responsible for maintenance of this cell phenotype as well as metastasis or poor response to irradiation, chemotherapeutic drugs or other agents [24]. This cell phenotype and behavior are, in part, controlled by miR-NAs [3].

Childs et al. proposed that low level miR-205 is associated with loco-regional recurrence independent of disease severity at diagnosis and treatment. Moreover, these researchers postulated that combination of low levels of let-7d and miR-205 expression is associated with poor survival and that these two miRNAs can be used as prognostic markers of HNSCC [7]. However, these results have not been validated using different/larger cohort of patients and the molecular basis of this observation has not been elucidated. The present study used TCGA data and analyzed 307 patients with HNSCC to investigate the diagnostic utility of let-7d and miR-205. The present results contradicted those reported by Childs and colleagues. In the present study, patients with low let-7d and high miR-205 levels had significantly better survival than patients with opposite phenotype - high let-7d and low miR-205 levels. Due to differences in survival between these groups, it was checked whether they differ in terms of other clinical features. We observed, that in the low let-7d and high miR-205 expression level group, there was a lower percentage of more advanced cancers.

It should be noted, that unexpectedly, the small group of patients with low let-7d and miR-205 expression levels was characterized by the best survival. However, these patients comprised 4% of all analyzed cases and due to the small number of cases, this group was not further analyzed by us.

Our analysis confirmed that both let-7d and miR-205 were frequently upregulated or remained unchanged in HNSCC. Other studies have also indicated that let-7d and miR-205 are upregulated in HNSCC [6] and in other cancers [4, 18, 19].

We observed, that patients with higher levels of let-7d and lower levels of miR-205 had worse outcome and more advanced cancers than the group with low let-7d and high miR-205 expression levels.
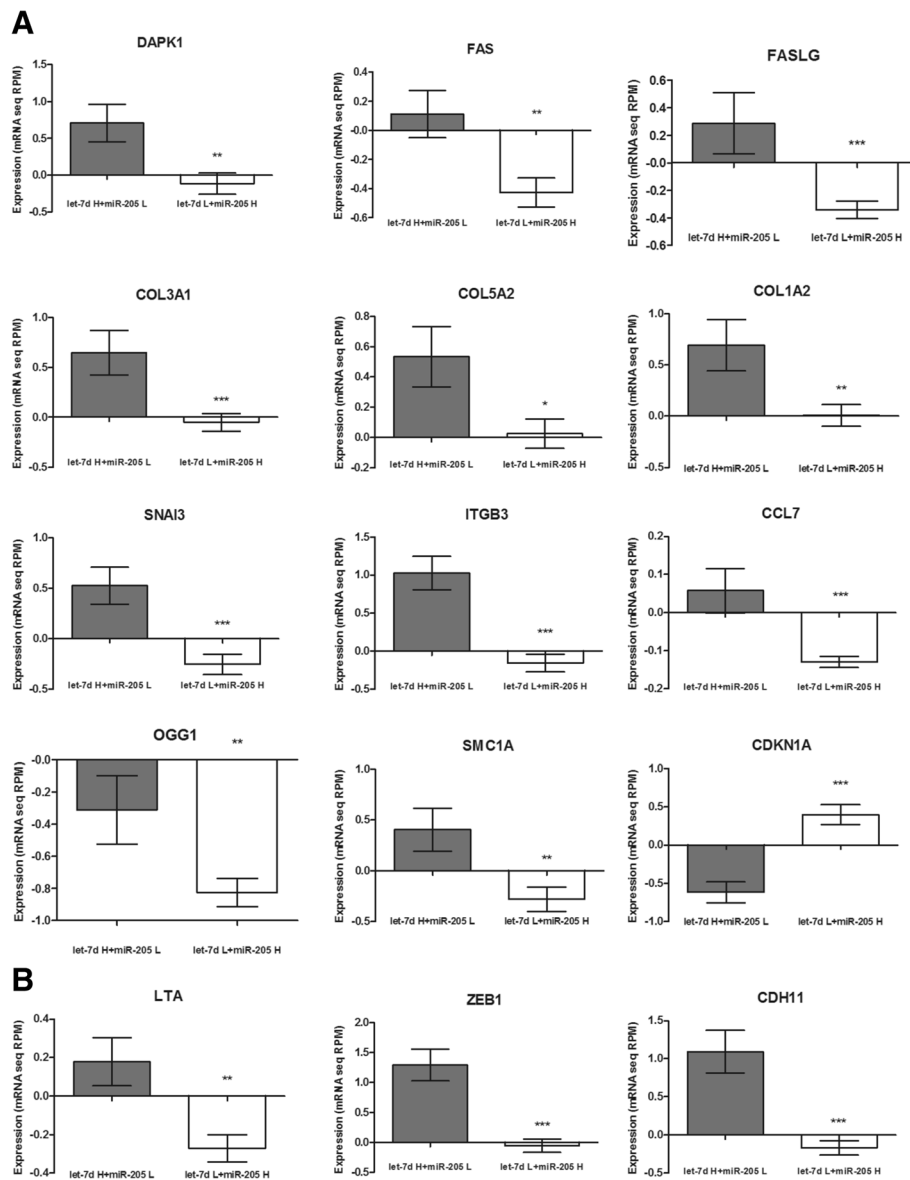
**Fig. 6** Expression of predicted targets for let-7d (A) and miR-205 (B) in HNSCC patient groups with high let-7d and low miR-205 expression levels (let-7d H + miR-205 L) and low let-7d and high miR-205 expression levels (let-7d L + miR-205 H); unpaired T-test; mean of mRNA expression z-Scores (RNA Seq V2 RSEM; z = 2) with SEM; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

More advanced tumors (higher N-stage and grade) that generally metastasize to blood and lymph vessels had higher levels of let-7d. Similarly, Hilly et al. found that higher expression of let-7d (and other members of the let-7 family) is associated with more aggressive oral tongue carcinoma in young patients [25]. Based on an in vitro model, Di Fiore et al. demonstrated that let-7d also acts as an oncogene [10]. Our previous study using a FaDu cell line (squamous cell carcinoma of the hypopharynx) with upregulated let-7d expression showed that higher levels of let-7d causes more irradiation-resistant cells but not chemotherapeutic drug-resistant cells [9].

In contrast, down-regulation of let-7d promotes EMT and higher self-renewal of cancer-initiating cells as well as influences the chemoresistant property of oral cancer cells [13]. However, the present results based on TCGA data indicated that let-7d can act as oncogene.

MiR-205, the second analyzed microRNA may function as a tumor suppressor or an oncogene [18, 19]. miR-205 is an epithelial marker [16, 17], and it is a well known modulator of the EMT process [15]. Jamali et al. performed a meta-analysis and showed that down-regulation of miR-205 is associated with poor prognosis in HNSCC [26]. Our results demonstrated that high expression of

miR-205 was related to less aggressive tumors, less metastasis to blood, less metastasis to lymph vessels and lower ability of perineural invasion. The present findings also indicated that patients with high miR-205 expression had no dissection of neck lymph nodes.

Clinical and pathological parameters indicate specific, different phenotypes between these groups. To check this, we decided to analyze some important processes such as: cell cycle, apoptosis, EMT, cancer-initiating cells, metastasis, irradiation response and drug response between these groups. Next, we checked the role of let-7d and miR-205 in these processes. We observed differences in the expression of genes associated with the analyzed processes between patients with higher levels of let-7d and lower levels of miR-205 than the group with low let-7d and high miR-205 expression levels.

In the group of patients with low let-7d and high miR-205, the analysis of predicted targets for let-7d showed that the predicted targets, with the exception of CDKN1A (p21), were down-regulated and that these genes were related to apoptosis, EMT, cell cycle, cell proliferation and DNA damage and repair. Llanos et al. indicated that HNSCC patients with p21- and phosphoS6-positive tumors present a better disease-specific survival [27]. Moreover, patients with p21-positive tumors have better overall survival than those with p21-negative tumors [28]. However, immunohistochemical staining of p21 and Ki-67 has shown that coexpression of p21/Ki-67 is a strong negative prognostic factor in HNSCC and may be important in patients treated by primary radiotherapy [29]. p21 can function as a suppressor or oncogene, and it is an example of an antagonistic duality molecule, in which function is determined by particular cellular context [30]. In contrast, the present results suggested that let-7d influences a specific phenotype of HNSCC through direct regulation of CDKN1A and results in better patient survival.

In the analyzed processes we observed three miR-205 targets - LTA, ZEB1 and CDH11, which were down-regulated in the group of patients with low let-7d and high miR-205 compared to the group of patients with high let-7d and low miR-205 expression levels. The LTA gene-encoded tumor necrosis factor-beta (TNF-β), which is a member of the large family of cytokines, is down-regulated in HNSCC compared to normal samples, but TGF receptors RI and RII are upregulated in HNSCC [31]. Buhrmann et al. reported that TNF-β increases the capacity of cell survival and invasion as well as positively influences the EMT process and maintenance of the cancer-initiating cell-like phenotype in colon cancer. Moreover, TNF-β promotes the chemoresistance of colon cancer cells to 5-FU [32].

Based on the HNSCC samples, the high expression of ZEB1 and ZEB2 was associated with advanced and metastatic cancer, and high levels of ZEB1 and ZEB2 predicted poor outcome. ZEB1 and ZEB2 are key modulators of cancer-initiating cell properties in HNSCC, EMT process, metastasis and cisplatin resistance [33]. Kurihara et al. showed that BMI1 and ZEB1 are important factors for promotion of EMT and invasion of tongue cancer. Expression of ZEB1 positively correlates with vimentin, but no significant correlation between ZEB1 and E-cadherin has been identified [34]. The present analysis indicated that the group of patients with better survival had lower levels of cadherin-11 (CDH11). Ma et al. showed that the expression of CDH6, CDH11 and CD44 is upregulated in cancer tissue compared to normal mucosa and is highly increased in OSCC patients with lymph node metastasis. Moreover, OSCC patients with high coexpression of CDH6, CDH11 and CD44 exhibit lower disease-specific survival time, and these molecules may be used as biomarkers of metastases and prognosis [35]. In addition, downregulation of CDH11 promotes proliferation and enhances invasion, and CDH11 has tumor suppressor function. However, overexpression of CDH11 is cancer-specific [36].

## Conclusion
In conclusion, the present study clearly shows that patients with low let-7d and high miR-205 expression levels had less advanced cancers and better survival compared to patients with high let-7d and low miR-205 expression levels. These results contrasted the model presented by Childs and colleagues [7], but the present observations were based on a larger group of patients and were consistent with other studies that describe let-7d as an oncogene [9, 10]. Down-regulation of let-7d and overexpression of miR-205 create a unique cell phenotype with different behavior that influences patient survival compared to cells with upregulated let-7d and down-regulated miR-205. Thus, let-7d and miR-205 are responsible for the observed phenomenon by direct regulation of CDKN1A (let-7d) and by regulation of LTA, ZEB1 and CDH11 (miR-205). However, other mechanisms may better explain the observed results. In conclusion, let-7d and miR-205 are good candidates for new biomarkers in the personalization of HNSCC treatment.

UTR: Untranslated region; ZEB1: Zinc finger E-box binding homeobox 1; ZEB2: Zinc finger E-box binding homeobox 2

## Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Disclosure policy
All authors read and approved the final manuscript. The authors declare that there is no conflict of interest regarding the publication of this paper.

## Authors' contributions
TK and KG have contributed equally to this work. All authors read and approved the final manuscript.

## Ethics approval and consent to participate
Study is based on analysis of available TCGA data from cBioportal.org web site and does not need any ethics committee's agreement, and does not violate the rights of other persons or institutions.

## Consent for publication
Not applicable.
Study is based on analysis of available TCGA data.

## Competing interests
The authors declare that they have no competing interests.

# Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## Author details
[1]Laboratory of Cancer Genetics, Greater Poland Cancer Centre, 15th Garbary Street, room 5025, 61-866 Poznan, Poland. [2]Department of Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland. [3]Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warszawa, Poland.

## References
1.  Cohen N, Fedewa S, Chen AY. Epidemiology and Demographics of the Head and Neck Cancer Population. Oral Maxillofac Surg Clin North Am. 2018;30(4):381–95.
2.  Marur S, Forastiere AA. Head and neck cancer: changing epidemiology, diagnosis, and treatment. Mayo Clin Proc. 2008 Apr;83(4):489–501.
3.  Irani S. miRNAs signature in head and neck squamous cell carcinoma metastasis: a literature review. J Dent (Shiraz). 2016 Jun;17(2):71–83.
4.  Kolenda T, Przybyła W, Teresiak A, Mackiewicz A, Lamperska KM. The mystery of let-7d- a small RNA with great power. Contemp Oncol (Pozn). 2014;18(5):293–301.
5.  Kolenda T, Teresiak A, Kapałczyńska M, Przybyła W, Zajączkowska M, Bliźniak R, Lamperska K. Let-7d and miR-18a as biomarkers of head and neck cancers. Lett Oncol Sci. 2015;12:37–47.
6.  Lamperska KM, Kozlowski P, Kolenda T, Teresiak A, Blizniak R, Przybyla W, et al. Unpredictable changes of selected miRNA in expression profile of HNSCC. Cancer Biomark. 2016;16(1):55–64.
7.  Childs G, Fazzari M, Kung G, Kawachi N, Brandwein-Gensler M, McLemore M, et al. Low-level expression of microRNAs let-7d and miR-205 are prognostic markers of head and neck squamous cell carcinoma. Am J Pathol. 2009 Mar; 174(3):736–45.
8.  Wei Y, Liu G, Wu B, Yuan Y, Pan Y. Let-7d inhibits growth and metastasis in breast cancer by targeting Jab1/Cops5. Cell Physiol Biochem. 2018 Jul 5; 47(5):2126–35.
9.  Lamperska KM, Kolenda T, Teresiak A, Kowalik A, Kruszyna-Mochalska M, Jackowiak W, et al. Different levels of let-7d expression modulate response of FaDu cells to irradiation and chemotherapeutics. PLoS One. 2017 Jun 30;12(6).
10. Di Fiore R, Drago-Ferrante R, Pentimalli F, Di Marzo D, Forte IM, Carlisi D, et al. Let-7d miRNA shows both antioncogenic and oncogenic functions in osteosarcoma-derived 3AB-OS cancer stem cells. J Cell Physiol. 2016 Aug; 231(8):1832–41.
11. Boyerinas B, Park SM, Murmann AE, Gwin K, Montag AG, Zillhardt M, et al. Let-7 modulates acquired resistance of ovarian cancer to taxanes via IMP-1-mediated stabilization of multidrug resistance 1. Int J Cancer. 2012 Apr 15; 130(8):1787–97.
12. Sun H, Ding C, Zhang H, Gao J. Let-7 miRNAs sensitize breast cancer stem cells to radiation-induced repression through inhibition of the cyclin D1/Akt1/Wnt1 signaling pathway. Mol Med Rep. 2016 Oct;14(4):3285–92.
13. Chang CJ, Hsu CC, Chang CH, Tsai LL, Chang YC, Lu SW, et al. Let-7d functions as novel regulator of epithelial-mesenchymal transition and chemoresistant property in oral cancer. Oncol Rep. 2011 Oct;26(4):1003–10.
14. Wang H, Chen B, Duan B, Zheng J, Wu X. miR-205 suppresses cell proliferation, invasion and metastasis via regulation of the PTEN/AKT pathway in renal cell carcinoma. Mol Med Rep. 2016 Oct;14(4):3343–9.
15. Mayoral-Varo V, Calcabrini A, Sánchez-Bailón MP, Martín-Pérez J. miR-205 inhibits stem cell renewal in SUM159PT breast cancer cells. PLoS One. 2017;12(11).
16. Fletcher AM, Heaford AC, Trask DK. Detection of metastatic head and neck squamous cell carcinoma using the relative expression of tissue-specific mir-205. Transl Oncol. 2008 Dec;1(4):202–8.
17. Kurihara K, Isobe T, Yamamoto G, Tanaka Y, Katakura A, Tachikawa T. Expression of BMI1 and ZEB1 in epithelial-mesenchymal transition of tongue squamous cell carcinoma. Oncol Rep. 2015 Aug;34(2):771–8.
18. Orang AV, Safaralizadeh R, Hosseinpour Feizi MA. Insights into the diverse roles of miR-205 in human cancers. Asian Pac J Cancer Prev. 2014;15(2):577–83.
19. Qin AY, Zhang XW, Liu L, Yu JP, Li H, Wang SZ, et al. MiR-205 in cancer: an angel or a devil? Eur J Cell Biol. 2013 Feb;92(2):54–60.
20. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013 Apr 2;6(269):pl1.
21. Li JH, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interactionnetworks from large-scale CLIP-Seq data. Nucleic Acids Res. 2014 Jan;42(Database issue):D92–7.
22. Wong N, Wang X. miRDB: an online resource for microRNA target prediction and functional annotations. Nucleic Acids Res. 2015;43(D1):D146–52.
23. Kolenda T, Przybyła W, Kapałczyńska M, Teresiak A, Zajączkowska M, Bliźniak R, Lamperska KM. Tumor microenvironment - unknown niche with powerful therapeutic potential. Rep Pract Oncol Radiother. 2018 May-Jun;23(3):143–53.
24. da Silva-Diz V, Lorenzo-Sanz L, Bernat-Peguera A, Lopez-Cerda M, Muñoz P. Cancer cell plasticity: impact on tumor progression and therapy response. Semin Cancer Biol. 2018.
25. Hilly O, Pillar N, Stern S, Strenov Y, Bachar G, Shomron N, Shpitzer T. Distinctive pattern of let-7 family microRNAs in aggressive carcinoma of the oral tongue in young patients. Oncol Lett. 2016;12(3):1729–36.
26. Jamali Z, Asl Aminabadi N, Attaran R, Pournagiazar F, Ghertasi Oskouei S, Ahmadpour F. MicroRNAs as prognostic molecular signatures in human head and neck squamous cell carcinoma: a systematic review and meta-analysis. Oral Oncol. 2015 Apr;51(4):321–31.
27. Llanos S, García-Pedrero JM, Morgado-Palacin L, Rodrigo JP, Serrano M. Stabilization of p21 by mTORC1/4E-BP1 predicts clinical outcome of head and neck cancers. Nat Commun. 2016;7:10438.
28. Chernock RD, Wang X, Gao G, Lewis JS Jr, Zhang Q, Thorstad WL, El-Mofty SK. Detection and significance of human papillomavirus, CDKN2A(p16) and CDKN1A(p21) expression in squamous cell carcinoma of the larynx. Mod Pathol. 2013 Feb;26(2):223–31.
29. Fischer CA, Jung M, Zlobec I, Green E, Storck C, Tornillo L, et al. Co-overexpression of p21 and Ki-67 in head and neck squamous cell carcinoma relative to a significantly poor prognosis. Head Neck. 2011 Feb;33(2):267–73.
30. Gartel AL. p21(WAF1/CIP1) and cancer: a shifting paradigm? Biofactors. 2009 Mar-Apr;35(2):161–4.
31. von Biberstein SE, Spiro JD, Lindquist R, Kreutzer DL. Enhanced tumor cell expression of tumor necrosis factor receptors in head and neck squamous cell carcinoma. Am J Surg. 1995 Nov;170(5):416–22.

Kolenda *et al. Journal of Biomedical Science*     (2019) 26:17

Page 11 of 11

32.  Buhrmann C, Yazdi M, Popper B, Shayan P, Goel A, Aggarwal BB, Shakibaei M. Resveratrol Chemosensitizes TNF-β-induced survival of 5-FU-treated colorectal Cancer cells. Nutrients. 2018;10(7).
33.  Chu PY, Hu FW, Yu CC, Tsai LL, Yu CH, Wu BC, et al. Epithelial-mesenchymal transition transcription factor ZEB1/ZEB2 co-expression predicts poor prognosis and maintains tumor-initiating properties in head and neck cancer. Oral Oncol. 2013;49:34–41.
34.  Kimura S, Naganuma S, Susuki D, Hirono Y, Yamaguchi A, Fujieda S, Sano K, Itoh H. Expression of microRNAs in squamous cell carcinoma of human head and neck and the esophagus: miR-205 and miR-21 are specific markers for HNSCC and ESCC. Oncol Rep. 2010;23:1625–33.
35.  Ma C, Zhao JZ, Lin RT, Zhou L, Chen YN, Yu LJ, et al. Combined overexpression of cadherin 6, cadherin 11 and cluster of differentiation 44 is associated with lymph node metastasis and poor prognosis in oral squamous cell carcinoma. Oncol Lett. 2018;15(6):9498–506.
36.  Piao S, Inglehart RC, Scanlon CS, Russo N, Banerjee R, D'Silva NJ. CDH11 inhibits proliferation and invasion in head and neck cancer. J Oral Pathol Med. 2017 Feb;46(2):89–97.