A pilot study to evaluate the expression of microRNA-let-7a in patients with intestinal-type sinonasal adenocarcinoma

FEDERICO MARIA GIOACCHINI[1], ARIANNA DI STADIO[2], PIETRO DE LUCA[3], ANGELO CAMAIONI[4], ANNALISA PACE[5], GIANNICOLA IANNELLA[5], CORRADO RUBINI[6], MARCO SANTARELLI[6], MARCO TOMASETTI[7], ALFONSO SCARPA[8], FABIOLA OLIVIERI[9,10] and MASSIMO RE[1]

[1]Ear, Nose and Throat Unit, Department of Clinical and Molecular Sciences, Polytechnic University of Marche, Ancona Joint Hospitals, I-60020 Ancona; [2]Gian Filippo Ingrassia Department, Otolaryngology Unit, University of Catania, I-95121 Catania; [3]Department of Otolaryngology, Fatebenefratelli Isola Tiberina-Gemelli Hospital, I-00100 Rome; [4]Head and Neck Department, San Giovanni-Addolorata Hospital, I-00189 Rome; [5]Department of Sense Organs, University La Sapienza of Rome, I-00161 Rome; [6]Pathology and Histopathology Division, Department of Biomedical Sciences and Public Health; [7]Department of Clinical and Molecular Sciences, Section of Occupational Medicine, Polytechnic University of Marche, I-60020 Ancona; [8]Department of Medicine and Surgery, University of Salerno, I-84084 Fisciano; [9]Clinic of Laboratory and Precision Medicine, IRCCS INRCA, I-60121 Ancona; [10]Department of Clinical and Molecular Sciences, Università Politecnica Delle Marche, I-60126 Ancona, Italy

Received July 10, 2023; Accepted October 25, 2023

DOI: 10.3892/ol.2023.14202

Abstract. Despite its histological resemblance to colorectal adenocarcinoma, there is little information about the molecular events involved in the pathogenesis of intestinal-type sinonasal adenocarcinoma (ITAC). The present study investigated the possible role and clinical value of microRNA (miR)-let-7a, a head and neck squamous cell carcinoma-related miR, in a well-characterized and homogeneous cohort of patients with ethmoidal ITAC associated with occupational exposure, treated by primary surgery. miR-let-7a expression levels were analyzed in 23 pairs of ethmoidal ITAC and adjacent normal formalin-fixed paraffin-embedded tissues by reverse transcription-quantitative PCR. The expression was evaluated in tumor and healthy tissues according to: Tumor grade (G) of differentiation and extension, and pTNM stage, and presence/absence of recurrence. Comparisons within and between groups were performed using two-tailed Student's paired t-test and one-way ANOVA with Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference. miR-let-7a expression in ethmoidal ITAC tissues was significantly lower than that in adjacent normal tissues (P<0.05; mean expression level ± SD, 1.452707±1.4367189 vs. 4.094017±2.7465375). miR expression varied with pT stage. miR-let-7a was downregulated (P<0.05) in advanced stages (pT3-pT4) compared with earlier stages (pT1-pT2). Furthermore, downregulation of miR-let-7a in ITAC was associated with poorly-differentiated (G3) cancer (P<0.05). No other associations were observed between miR-let-7a expression and the other clinicopathological parameters, including disease-free survival. In conclusion, downregulation of miR-let-7a in ITAC was associated with advanced-stage (pT3 and pT4) and poorly-differentiated (G3) disease, suggesting that the mutation of this gene, combined with additional genetic events, could serve a role in ITAC pathogenesis.

Introduction

Malignant neoplasms of the nasal cavity and paranasal sinuses represent 0.2% of all human primary malignant tumors, and their incidence is about 0.1-1.4 new cases/year/100.000 inhabitants (1,2). Among primary malignant neoplasms of the sinonasal tract nasal, sinonasal adenocarcinomas account for 10-20% (3); the majority of them show a salivary gland origin, while others show histological features resembling those of colon adenocarcinoma. One particular subtype of sinonasal adenocarcinoma was named intestinal-type adenocarcinoma (ITAC), which is responsible for less than 4% of total malignancies in this region (4), and can occur sporadically or associated with specific workers' categories that are exposed to hardwood and leather dust (5): in fact, these high-risk individuals show an approximately 500-fold higher incidence (6,7), and ITACs arising in subjects with occupational dust exposure are more often diagnosed in men, and show a significant propensity to develop in the ethmoid sinuses (8-10). In addition, the ITACs connected with occupational exposure (hardwood dust, leather dust) are preceded by the intestinal metaplasia of the respiratory mucosal tract.

Correspondence to: Professor Arianna Di Stadio, Gian Filippo Ingrassia Department, Otolaryngology Unit, University of Catania, Via di Santa Sofia 75, I-95121 Catania, Italy
E-mail: ariannadistadio@hotmail.com

Key words: sinonasal cancer, intestinal-type sinonasal adenocarcinoma, microRNA, intestinal-type

ITAC represent a clinically aggressive entity that is typically associated with a tendency to recur locally, a low incidence of distant metastases, and an overall mortality of approximately 53% (10). Several studies reported how the most important prognosticators in patients with ITAC are the histopathological grading, and the pT classification (9-12). Surgery combined with postoperative radiotherapy (RT) and, in some cases, with adjuvant chemotherapy (CH) is the best treatment option (13).

Although the classic prognostic factors maintain a great utility in predicting the clinical behavior of ITAC (13,14), nevertheless it is not clear why some ITACs present a more aggressive behavior in comparison to others with the same type of features in terms of histological subtype and clinical stage (15-25). In the last ten years, information about the molecular mechanisms involved in the pathogenesis of head and neck squamous-cell carcinomas (HNSCC) are rapidly increasing (26-36); recently, some authors showed that epigenetic alterations have a critical role in HNSCC carcinogenesis (37-39). MicroRNAs (miRNAs), a type of small non-protein-coding RNA molecules that modulate the expression of target genes, operating as gene expression repressors at post-transcriptional level, and affecting the translation or causing the degradation of mRNA targets, are now considered as crucial components of the epigenome, orchestrating events ranging from organogenesis to immunity, and they are known to be important in influencing the origin of many diseases, including malignant tumors (40-54).

To achieve further knowledge about the phenotype and possible mechanisms of ethmoidal ITAC development, for the first time we investigated the role and the prognostic value of miR-let-7a, a head and neck squamous-cell carcinoma (HNSCC) related microRNA (53-55) in a well-characterized and homogeneous cohort of patients affected by primary ethmoidal ITAC, associated with occupational exposure and treated by primary surgery (56,57).

Materials and methods

Patients and specimen selection. This retrospective cohort study analyzed consecutively the medical charts of all patients with primary ITAC, treated by surgery with curative intent at the Department of Otorhinolaryngology, Umberto I University General Hospital (Marche Polytechnic University, Ancona, Italy) between January 2000 and January 2010. From the medical records, the following data were collected: age at the diagnosis, gender, occupational history, site of the tumor, postoperative staging (pTNM classification), histological findings, disease-free survival (DFS), and overall survival (OS).

Inclusion criteria: complete clinical data, 5 follow-up years at least, uniformity of histological differentiation throughout the tumor sample, and the availability of normal formalin-fixed paraffin-embedded (FFPE) tissue samples.

Exclusion criteria: patients with previous or synchronous second malignancies, or patients who underwent previous radiation therapy or chemotherapy, or patients who died of postoperative complications. As additional, specifical exclusion criteria were applied regarding the surgical approach for removing the tumors as previously described and suggested in the literature (13,58,59). Absolute contraindications for an endoscopic approach were erosion of nasal bones or floor of the nasal cavity, extensive involvement of the nasal pathway (except the nasolacrimal duct), infiltration of the walls of the maxillary sinus (except the medial one), and invasion of the orbital content.

Length of survival was calculated from the date of surgery to the date of the latest clinical follow-up, or to the date of death by disease or other causes. Representative tissue blocks were collected from the archives of the section of Pathology Department of Marche Polytechnic University (Ancona, Italy). The paired samples from tumor tissues and adjacent normal tissues were obtained from patients with ITAC who had undergone surgical resection. The diagnosis and assessment of the histological grading (G, from G1 to G3) of tumor differentiation were made on 4-6 μm-thick paraffin tissue sections stained with conventional hematoxylin and eosin according to WHO Classification of Head and Neck Tumors (56).

In all the cases, ITAC tumor diagnosis was confirmed, as described by Barnes (10).

The identification of the anatomical site of the tumor (T, from T1 to T4), nodal involvement (N0, N1, N2), and clinical-pathologic stage were determined according to AJCC/UICC TNM classification (7th edition) (57).

Written informed consent was obtained at the time of surgery from each patient included in the study, and samples were processed after approval of the Ethical Committee of the Marche Regional Hospital, Ancona, Italy, Rec. no. 501 of November 29, 2011.

Patient cohort and workup. The clinical data were collected prospectively from patients, then updated retrospectively after the follow-up review. All patients underwent complete clinical examination and were staged by multiplanar CT and by contrast-enhanced MRI (or contrast-enhanced CT whenever an MRI could not be obtained). After imaging evaluation, a biopsy with the patient under local anesthesia was performed. Treatment planning was discussed by the local multidisciplinary team.

Surgery. All patients were treated by surgery that was, depending on the position and the extension of the tumor or a craniofacial and cranioendoscopic resection or a transnasal endoscopic approach with or without transnasal craniectomy. In all cases we used surgical techniques already described in the literature (13,58,59).

Patients who had absolute contraindication to endoscopic approach as describe in the exclusion criteria performed traditional open surgical approach (13).

Histological evaluation. Tissue blocks were collected, and the histological slides were examined by a senior pathologist (C.R.) to confirm the diagnosis of ITAC and to assess the grade of histopathological differentiation of each tumor, according to WHO criteria [56], as follows: G1=well-differentiated, G2=moderately differentiated, and G3=poorly-differentiated.

Surgical and histological reports were analyzed, and all the lesions were retrospectively staged according to the TNM classification (57).

Adjuvant therapy. Although advanced stage, poor differentiation, and presence of positive surgical margins were the main considered factors, the indication for adjuvant RT and /or CHT was discussed for each patient by the multidisciplinary team,

Table I. Overview of the clinical and pathological characteristics of patients with primary ethmoidal intestinal-type sinonasal adenocarcinoma.

Patient	Age, years	Sex	TNM stage	Grade	CH	CHT	RT
1	70	M	T2N0M0	3	Yes	No	No
2	55	M	T3N0M0	2	Yes	No	Yes
3	67	M	T3N0M0	2	Yes	No	Yes
4	60	F	T1N0M0	1	Yes	No	No
5	62	M	T3 N0M0	3	Yes	No	Yes
6	54	M	T3N0M0	2	Yes	No	Yes
7	54	M	T3N0M0	3	Yes	No	Yes
8	74	M	T3N0M0	1	Yes	No	Yes
9	74	M	T2N0M0	2	Yes	No	Yes
10	58	M	T1N0M0	1	Yes	No	No
11	74	M	T2N0M0	3	Yes	No	Yes
12	70	M	T2N0M0	2	Yes	No	Yes
13	72	M	T3N0M0	1	Yes	No	Yes
14	68	M	T3N0M0	3	Yes	No	Yes
15	77	M	T3N0M0	2	Yes	No	Yes
16	67	M	T3N0M0	3	Yes	No	Yes
17	77	M	T2N0M0	2	Yes	No	No
18	65	M	T3N0M0	3	Yes	No	Yes
19	63	M	T2N0M0	1	Yes	No	Yes
20	61	F	T1N0M0	1	Yes	No	No
21	65	M	T2N0M0	2	Yes	No	No
22	61	M	T4aN0M0	3	Yes	No	Yes
23	76	M	T4bN0M0	3	Yes	No	Yes

CH, chemotherapy; CHT, adjuvant chemotherapy; F, female; M, male; RT, radiotherapy.

also considering age, comorbidities, previous treatment and, especially for low-stage ITAC, the availability of the patient for adequate follow-up.

Follow-up. All patients were followed according to our institutional protocols by endoscopic evaluation and MRI every 2 and 4 months, respectively, during the first year, both endoscopic evaluation and MRI every 6 months until the fifth year, and clinical evaluation and MRI yearly thereafter; this follow-up protocol was the same applied in literature on large sample of patients (59).

miRNA detection by reverse transcription-quantitative PCR (RT-qPCR). Expression levels of miR-let-7a were measured in 23 FFPE samples of ethmoidal ITAC and in the corresponding adjacent healthy normal tissues considered as control (CTR).

The expression of miR-let-7a was also evaluated according to: tumor grade of differentiation G (G1 or G2 vs. G3), tumor extension (T1 or T2 vs. T 3 or T4), tumor stage (I or II vs. III or IV), and presence or absence of recurrence.

Total RNA was extracted from FFPE samples using FFPE RNA/DNA purification Kit (Norgen, Canada). MiRNAs were quantified by RT-qPCR using TaqMan miRNA assays (Applied Biosystems, Foster City, CA, USA) according to the Manufacturer's protocol. Data were analyzed with the iCycler (Bio-Rad Laboratories, Segrate, Milan, Italy) with an automatic setting for assigning the baseline. RT-qPCR data were standardized to RNU48.

The $2^{-\Delta\Delta Cq}$ method was used for quantification (60). Relative miR expression obtained from RT-qPCR was calculated using Ct (cycle threshold), i.e., the fractional cycle number where a fluorescent signal reaches the detection threshold. Levels of miRs expressed with reference to RNU48 were turned into linear form using the formula 2-DCt, DCt=Ct miR-X-Ct RNU), and reported as arbitrary units (a.u.). Ct values from RT-qPCR assays >35 were considered as not expressed. The intra- and inter-assay variability of miR measurements were <5% and <10%, respectively.

Statistical analysis. Results are expressed as mean ± standard deviation (SD) or as median, quartile and confidence interval (CI). Comparisons between and among groups were performed using two-tailed Student's paired t-test (two groups) and analysis of variances (one-way ANOVA), followed by post-hoc Tukey analysis, respectively. $P<0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS statistical package (SPPS Inc. Chicago, IL).

Results

Patient data. Overall, twenty-three patients met the inclusion criteria. Patient population consisted of twenty-one (91.3%)

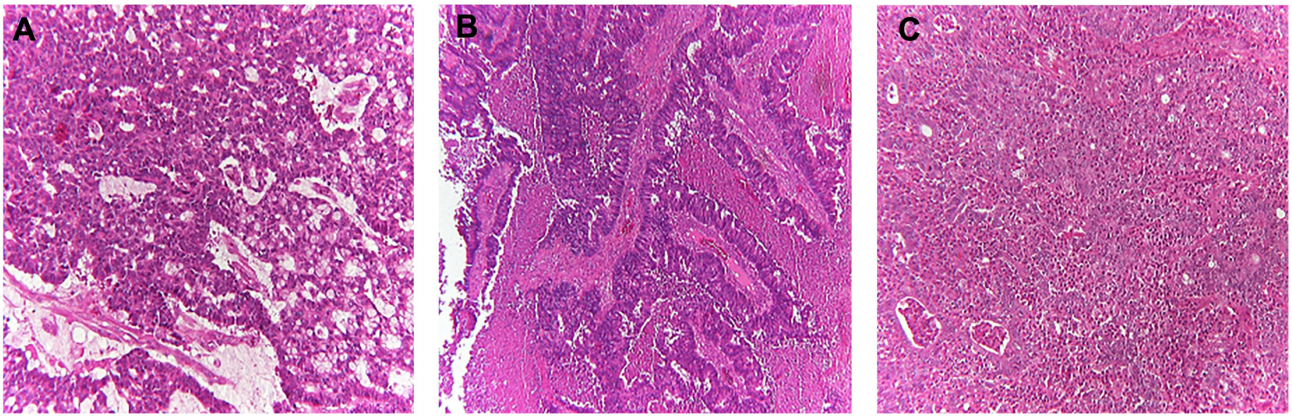

Figure 1. Histology of the tumor. (A) Well-differentiated (G1), (B) moderately differentiated (G2) and (C) poorly-differentiated (G3) intestinal-type sinonasal adenocarcinoma.

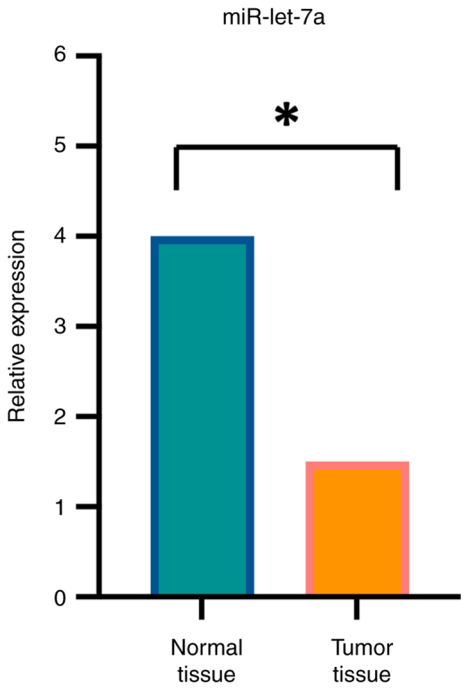

Figure 2. miR-let-7a expression in ethmoidal intestinal-type sinonasal adenocarcinoma tissues was significantly lower than that in adjacent normal tissues (mean expression level ± SD, 1.452707±1.4367189 vs. 4.094017±2.7465375). *P<0.05. miR, microRNA.

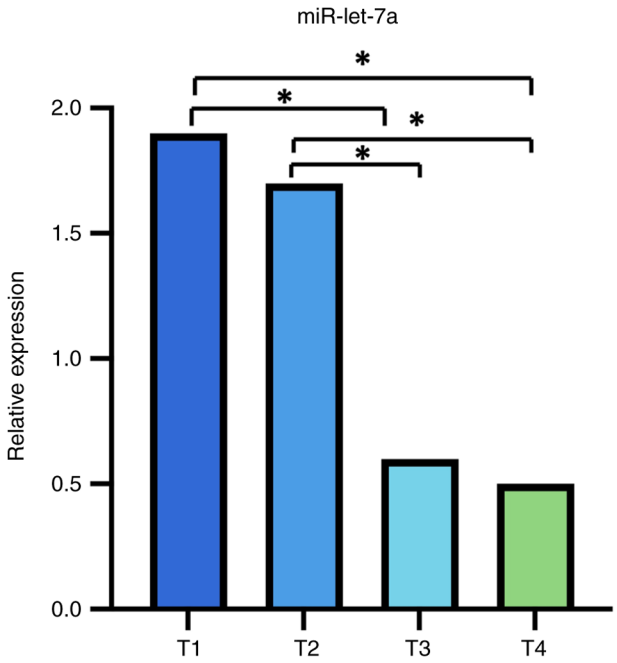

Figure 3. miR-let-7a varies with the T stage of the tumor, being downregulated in the more advanced stages (T3 and T4) compared with earlier stages (T1 and T2) [mean expression level ± SD, 0.584179392±0.581234561 (T3 and T4) vs. 1.926450065±1.627465375 (T1 and T2)]. *P<0.05. miR, microRNA.

males and two (8.7%) females, with a mean age of 66.3 years (range 54-77 yr). All the patients had a known history of occupational exposure to hardwood dust and the ITAC was in the ethmoid region in all cases, as confirmed by endoscopic and imaging (enhanced TC and/or MRI) evaluation. No patients presented clinical and radiological (cN) positive lymph nodes at diagnosis. The main clinicopathological features of the patients included in the study are summarized in Table I. No patients underwent selective neck dissection (ND).

Histological findings, post-operative staging (pTNM), and adjuvant therapy. The patients following pTNM classification were distributed as follows: three (13%) in stage I, seven (30.4%) in stage II, eleven (47.8%) in stage III, and two (8.7%) in stage IV.

Looking at the severity of the tumor (histological grade), the patients were distributed as follows: six patients affected by Grade 1(well-differentiated) (Fig. 1A), eight by Grade 2 (moderately differentiated) (Fig. 1B), and nine suffering from Grade 3 tumor (poorly differentiated) (Fig. 1C).

The immunohistochemistry analysis on the tissue showed that cases classified as ITAC had variable cellular appearance, and consisted of a mixture of tall columnar cells, atypical stratified cylindrical cells like the cells seen in conventional colorectal adenocarcinoma, goblet cells, and large round to polygonal non-descriptive epithelial cells.

None of the patients had a tumor 'within' (R1) or 'close to' (<1 mm, Rclose) the surgical margins. Nineteen (83%) of the patients underwent post-operative conventional RT on the primary site; nobody was treated by adjuvant CH.

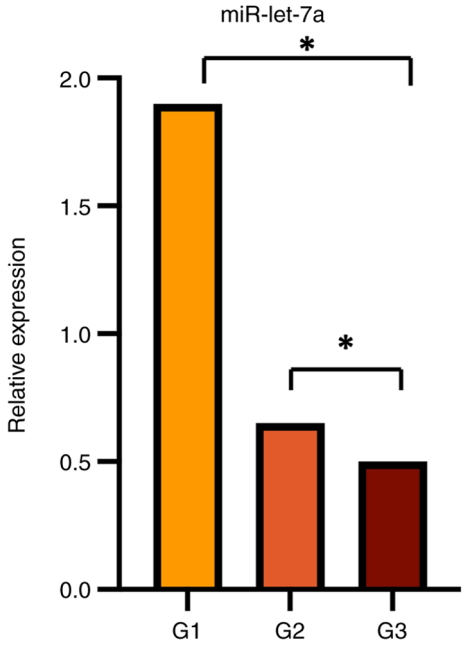

Figure 4. miR-let-7a expression in poorly differentiated ethmoidal ITAC tissues (G3) was significantly lower than that in well- and moderately differentiated ethmoidal ITAC tissues (G1 and G2) [mean expression level ± SD, 0.583269371±0.580274566 (G3) vs. 1.938231067±1.857467372 (G1 and G2)]. *P<0.05. ITAC, intestinal-type sinonasal adenocarcinoma; miR, microRNA.

miR-let-7a expression. miR-let-7a expression levels in ethmoidal ITAC tissues were significantly lower than in adjacent normal tissues (P<0.05) (Fig. 2). Moreover, miR-let-7a varies with the pT stage of the tumor, being lower-expressed in the more advanced stages (pT3-pT4) compared to earlier stages (pT1-pT2) (mean expression level ± SD; 1.452707±1.4367189 vs. 4.094017±2.7465375; P<0.05) (Fig. 3). Moreover, there was a statistically significant relationship between miR-let-7a down-expression and G3 histological grading (P<0.05) (Fig. 4). No other significant findings were found between miR-let-7a expression and the other clinicopathological parameters, including DFS.

Discussion

The results of our study showed, for the first time, that the overall expression levels of miR-let-7a were significantly down-regulated in tumor tissues as compared with the adjacent non-pathologic tissues (approximately three times lower in tumor samples than in normal tissue) (P<0.05). The down-regulation of miR-let-7a was associated with the pT stage, reaching the minimum levels of expression in pT3 and pT4 samples (P<0.05).

Furthermore, we found a down-regulation of miR-let-7a expression (P<0.05) in poorly-differentiated tumors (G3) compared with moderately- and well-differentiated tumors (G1-G2), indicating a potential role of miR-let-7a in the cell differentiation of ITACs. No other statistically significant variances were found looking at the up/down regulation of miR-let-7a related to other clinicopathological parameters, including DFS.

Despite their histological similarity to colorectal carcinomas, there is a scarce amount of data about the molecular events that are involved in ITAC pathogenesis. A wide number of tumorigenesis pathways have been identified in colorectal adenocarcinomas, and these pathways are related to mutation and inactivation of various oncogenes, tumor suppressor genes, and DNA mismatch repair genes, including K-ras, APC, p53, MLH1, and MSH2 (16,17,61).

Assuming that the morphological similarities to colorectal adenocarcinomas might reflect equivalent genetic alterations, the presence of activating mutations of Ras oncogenes and TP53 mutations in ITAC were investigated in many studies. TP53 mutations were found in 18-44% of mostly occupational ITACs, whereas K-Ras mutations were observed in 10-15% of ITACs. The results of these studies suggested that mutations of K-Ras and other Ras genes are relatively uncommon in ITAC and, similarly, TP53 mutations in ITACs have not been widely demonstrated (18,19,20,62,63). Other authors found that K-Ras mutation and C-erb-2 expression might be associated with a more aggressive behavior and poorer outcome (21).

Licitra *et al* (64) described two genetic ITACs subgroups, characterized by differences in TP53 mutational status or protein functionality, that significantly influence the pathological response to primary CH and, ultimately, the prognosis.

Perez-Ordonez and colleagues investigated the role of DNA mismatch repair (MMR) gene defects or disruptions of E-cadherin/β-catenin complex in ITAC by testing the immunohistochemical expression of the MMR gene products, E-cadherin and β-catenin, in a cohort of patients with sporadic ITACs, and they found that the nuclear expression of MLH1, MSH2, MSH3 and MSH6 were preserved in these tumors, suggesting that mutations or promoter methylation of MMR genes do not play a role in ITAC pathogenesis (65).

An interesting finding was performed by Kennedy and al., who found that sinonasal ITACs have a distinctive phenotype, with all the cases expressing CK20, CDX-2, villin, and most ITACs also expressing CK7. So the expression pattern of CK7, CK20, CDX-2, and villin positive may be used to distinguish these tumors from other non-ITACs of the sinonasal tract (66).

Furthermore, published data showed how miRNAs may have an important role in the carcinogenesis process because more than 50% of miRNAs were found in cancer-associated genomic regions or fragile sites (67,68). Some miRNAs seem to promote the cancer onset, while others inhibit the cell proliferation and survival. Basically, both miRNA classes are showing important connection with cancer development, being able to act like novel oncogenes or tumor suppressors, respectively (69,70). Measuring miRNAs expression as a biomarker may bring an important advantage in cancer diagnosis, prognosis and therapy. Also, concerning head and neck tumors there are many studies that have reported significant associations among miRNA profiles and patient survival (23,27-30,33-36).

At present time, due to the rarity of this type of cancer, there is still a lack of literature evaluating the expression of miRNAs in ITAC of the paranasal sinuses (25). Our research team previously investigated the status of MiR-126 and we found that it was reduced in ITACs compared with benign tumors, suggesting the potential role of this miRNA acting as a circulating biomarker for the detection of malignant transformation (25).

On the basis of these findings, to explore other pathways involved in the molecular pathogenesis of ITACs, in the current

study we tried to investigate the expression of miR-let-7a, which is an HNSCC related microRNA (53,55). To analyze the prognostic role of this miRNA, its expression levels were then retrospectively correlated with clinicopathological characteristics of the tumor itself and the patient's outcome to evaluate its independent prognostic relevance.

The let-7 gene family consists of 11 very closely related genes and miR-let-7a is currently the best characterized member. Recently the miR-let-7a expression was found to be reduced in different tumor model tissues, compared to adjacent healthy tissue and, therefore, miR-let-7a could probably act as a tumor suppressor miRNA (55,71-75).

miR-let-7a was found poorly expressed in many types of malignancies such as lung, colon, thyroid, and renal cancer (70-74). Furthermore, data from recent published works suggested that the down-regulation of let-7 miRNA family gene, targeting RAS oncogene, may be related with poor survival and relapse in surgical treated non-small cell lung cancer (53,71-72). The expression of miR-let-7a is also reduced in gastric cancer and relates to tumor cells differentiation degree. A xenograft model of mice showed that miR-let-7a acts as suppressor for the growth of gastric cancer *in vivo* and *in vitro* (76).

Long *et al* (55) evaluated the expression of miR-let-7a in a sample of 48 patients surgically treated for laryngeal primary carcinoma, and compared miR-let-7a levels among tumor tissue and adjacent healthy tissue. Results showed that in 37 out of 48 patients a statistically significant reduction in miR-let-7a expression level was present in all tumors with different clinical stages, compared with normal larynx tissues.

miR-let-7a could be a tumor suppressor in laryngeal cancer by inhibiting cell growth and inducing cell apoptosis. This action would be possible by down-regulating the protein expression of oncogenes such as RAS and c-MYC (target genes). Ras proteins are membrane associated GTPase, signaling proteins that regulate cellular growth and differentiation, while MYC is an evolutionarily conserved nuclear protein also involved in the control of cell proliferation and differentiation (55). An inverse correlation was observed among RAS/c-MYC protein and miR-let-7a expression in laryngeal tumor, suggesting that increased RAS and c-MYC protein expressions may be caused by the loss of miR-let-7a expression (55).

A down-regulation of tumor miR-let-7a suppressor family exists also in tumors of the Ewing's sarcoma family (ESFT). The mechanism by which miR-let-7a expression modulates the growth of ESFT has been shown to be mediated by its target gene HMGA2. An overexpression of miR-let-7a and the consequent repression of HMGA2 inhibit the tumorigenicity of ESFT cells (77).

The present study shows some limitations. In this work we did not consider the prognostic value of the expression of miR-let-7a, which will be the object of a second retrospective analysis. Second, although we tried to evaluate a homogeneous cohort of patients in terms of pTNM stage and treatment, our data were achieved from a retrospective cohort study and the patients cohort remains heterogeneous in some crucial clinical aspects (different pTNM classification, non-uniform surgical approach, mode, and effectiveness of complementary protocol treatment, follow-up). Moreover, the study did not conduct a sensitivity analysis due to the small sample size (n=43). Finally, understanding the connections between miRNAs deregulated in cancer and cellular signaling pathways involved in cancer was hindered by our limited knowledge of miRNA target recognition.

Despite these limitations, we presented a pilot study through highly standardized retrospective analysis of a single head and neck cancer institution.

In conclusion, this study provides the first evidence that a down-regulation of miR-let-7a in ethmoidal ITAC is associated with advanced stage (pT3 and pT4) disease, and with poorly differentiated tumors (G3). Our data suggest that the specific mutation of this gene, in combination with additional genetic events, could play a role in ITAC pathogenesis.

The analysis performed on a small sample of patients will necessarily be extended to a larger cohorts, and our single-institution results would require validation through a broader prospective and multicenter analysis.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

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

Authors' contributions

FMG and MR conceived the study. FMG wrote the first draft. ADS and PDL analyzed the data, defined the conclusions, revised the manuscript and helped write the manuscript. ADS and MS were involved in image curation by selecting the images and drawing the graphs. AC, AP, GI, AS and MS collected clinical data, and were involved in the analyses of data and definition of the results. CR, MT, MS and FO were involved in collection and analysis of histologic findings. FMG, FO and MR analyzed the data and participated in the definition of the conclusions. FMG and MR confirm the authenticity of all the raw data. All authors were involved in critical review of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethical Committee of the Marche Regional Hospital, Ancona, Italy, Rec. no. 501 of November 29, 2011. All patients provided written informed consent to participate in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Robin PD, Powell DJ and Stansbie JM: Carcinoma of the nasal cavity and paranasal sinuses: Incidence and presentation of different histological types. Clin Otolaryngol Allied Sci 4: 431-456, 1979.
2. Batsakis JG, Rice DH and Solomon AR: The pathology of head and neck tumors: Squamous and mucous-gland carcinomas of the nasal cavity, paranasal sinuses, and larynx, part 6. Head Neck Surg 2: 497-508, 1980.
3. Weber AL and Stanton AC: Malignant tumors of the paranasal sinuses: Radiologic, clinical, and histopathologic evaluation of 200 cases. Head Neck Surg 6: 761-776, 1984.
4. Lopez JI, Nevado M, Eizaguirre B and Perez A: Intestinal-type adenocarcinoma of the nasal cavity and paranasal sinuses. A clinicopathologic study of 6 cases. Tumori 76: 250-254, 1990.
5. Kleinsasser O and Schroeder HG: Adenocarcinomas of the inner nose after exposure to wood dust. Morphological findings and relationships between histopathology and clinical behavior in 79 cases. Arch Otorhinolaryngol 245: 1-15, 1988.
6. Leclerc A, Cortes MM, Gerin M, Luce D and Brugere J: Sinonasal cancer and wood dust exposure: Results from a case-control study. Am J Epidemiol 140: 340-349, 1994.
7. Stellman SD, Demers PA, Colin D and Boffetta P: Cancer mortality and wood dust exposure among participants in the American cancer society cancer prevention study-II (CPS-II). Am J Ind Med 34: 229-237, 1998.
8. Hadfield EH: A study of adenocarcinoma of the paranasal sinuses in woodworkers in the furniture industry. Ann R Coll Surg Engl 46: 301-319, 1970.
9. Klintenberg C, Olofsson J, Hellquist H and Sökjer H: Adenocarcinoma of the ethmoid sinuses. A review of 28 cases with special reference to wood dust exposure. Cancer 54: 482-488, 1984.
10. Barnes L: Intestinal-type adenocarcinoma of the nasal cavity and paranasal sinuses. Am J Surg Pathol 10: 192-202, 1986.
11. Franchi A, Gallo O and Santucci M: Clinical relevance of the histological classification of sinonasal intestinal-type adenocarcinomas. Hum Pathol 30: 1140-1145, 1999.
12. Fiaux-Camous D, Chevret S, Oker N, Turri-Zanoni M, Lombardi D, Choussy O, Frederic D, Jorissen M, de Gabory L, Malard O, *et al*: Prognostic value of the seventh AJCC/UICC TNM classification of intestinal-type ethmoid adenocarcinoma: Systematic review and risk prediction model. Head Neck 39: 668-678, 2017.
13. Maccariello G, Deganello A, Choussy O, Gallo O, Vitali D, De Raucourt D and Georgalas C: Endoscopic nasal versus open approach for the management of sinonasal adenocarcinoma: A pooled-analysis of 1826 patients. Head Neck 38 (Suppl 1): E2267-E2274, 2016.
14. Mills SE, Fechner RE and Cantrell RW: Aggressive sinonasal lesion resembling normal intestinal mucosa. Am J Surg Pathol 6: 803-809, 1982.
15. Batsakis JG, Mackay B and Ordonez NG: Enteric-type adenocarcinoma of the nasal cavity. An electron microscopic and immunocytochemical study. Cancer 54: 855-860, 1984.
16. McKinney CD, Mills SE and Franquemont DW: Sinonasal intestinal-type adenocarcinoma: Immunohistochemical profile and comparison with colonic adenocarcinoma. Mod Pathol 8: 421-426, 1995.
17. Franchi A, Massi D, Baroni G and Santucci M: CDX-2 homeobox gene expression. Am J Surg Pathol 27: 1390-1391, 2003.
18. Saber AT, Nielsen LR, Dictor M, Hagmar L, Mikoczy Z and Wallin H: K-ras mutations in sinonasal adenocarcinomas in patients occupationally exposed to wood or leather dust. Cancer Lett 126: 59-65, 1998.
19. Perez P, Dominguez O, Gonzalez S, Trivino A and Suarez C: Ras gene mutations in ethmoid sinus adenocarcinoma: Prognostic implications. Cancer 86: 255-264, 1999.
20. Wu TT, Barnes L, Bakker A, Swalsky PA and Finkelstein SD: K-ras-2 and p53 genotyping of intestinal-type adenocarcinoma of the nasal cavity and paranasal sinuses. Mod Pathol 9: 199-204, 1996.
21. Perrone F, Oggionni M, Birindelli S, Suardi S, Tabano S, Romano R, Moiraghi ML, Bimbi G, Quattrone P, Cantu G, *et al*: TP53, p14ARF, p16INK4a and H-ras gene molecular analysis in intestinal-type adenocarcinoma of the nasal cavity and paranasal sinuses. Int J Cancer 105: 196-203, 2003.
22. Re M, Magliulo G, Tarchini P, Mallardi V, Rubini C, Santarelli A and Lo Muzio L: p53 and BCL-2 over-expression inversely correlates with histological differentiation in occupational ethmoidal intestinal-type sinonasal adenocarcinoma. Int J Immunopathol Pharmacol 24: 603-609, 2011.
23. Gallo O, Franchi A, Fini-Storchi I, Cilento G, Boddi V, Boccuzzi S and Urso C: Prognostic significance of c-erbB-2 oncoprotein expression in intestinal-type adenocarcinoma of the sinonasal tract. Head Neck 20: 224-231, 1998.
24. Re M, Santarelli A, Mascitti M, Bambini F, Lo Muzio L, Zizzi A and Rubini C: Trail overexpression inversely correlates with histological differentation in intestinal-type sinonasal adenocarcinoma. Int J Surg Oncol 2013: 203873, 2013.
25. Tomasetti M, Re M, Monaco F, Gaetani S, Rubini C, Bertini A, Pasquini E, Bersaglieri C, Bracci M, Staffolani S, *et al*: MiR-126 in intestinal-type sinonasal adenocarcinomas: Exosomal transfer of MiR-126 promotes anti-tumour responses. BMC Cancer 18: 896, 2018.
26. Re M, Magliulo G, Ferrante L, Zizzi A, Santarelli A, Stramazzotti D, Lo Muzio L, Goteri G and Rubini C: p63 expression in laryngeal squamous cell carcinoma is related to tumor extension, histologic grade, lymph node involvement and clinical stage. J Biol Regul Homeost Agents 27: 121-129, 2013.
27. Re M, Zizzi A, Ferrante L, Stramazzotti D, Goteri G, Gioacchini FM, Olivieri F, Magliulo G and Rubini C: p63 and Ki-67 immunostainings in laryngeal squamous cell carcinoma are related to survival. Eur Arch Otorhinolaryngol 271: 1641-1651, 2014.
28. Re M, Ceka A, Rubini C, Ferrante L, Zizzi A, Gioacchini FM, Tulli M, Spazzafumo L, Sellari-Franceschini S, Procopio AD and Olivieri F: MiR-34c-5p is related to recurrence in squamous cell carcinoma. Laryngoscope 125: E306-E312, 2015.
29. Lucarini G, Zizzi A, Re M, Sayeed MA, Di Primio R and Rubini C: Prognostic implication of CEACAM1 expression in squamous cell carcinoma of the larynx: Pilot study. Head Neck 41: 1615-1621, 2019.
30. Re M, Gioacchini FM, Scarpa A, Cassandro C, Tulli M and Cassandro E: The prognostic significance of E-cadherin expression in laryngeal squamous cell carcinoma: A systematic review. Acta Otorhinolaryngologica Ital 38: 504-510, 2018.
31. Re M, Magliulo G, Gioacchini FM, Bajraktari A, Bertini A, Ceka A, Rubini C, Ferrante L, Procopio AD and Olivieri F: Expression levels and clinical significance of miR-21-5p, miR-let-7a, and miR-34c-5p in laryngeal squamous cell carcinoma. Biomed Res Int 2017: 3921258, 2017.
32. Re M, Gioacchini FM, Bajraktari A, Tomasetti M, Kaleci S, Rubini C, Bertini A, Magliulo G and Pasquini E: Malignant transformation of sinonasal inverted papilloma and related genetic alterations: A systematic review. Eur Arch Otorhinolaryngol 274: 2991-3000, 2017.
33. Gioacchini FM, Alicandri-Ciufelli M, Rubini C, Magliluo G and Re M: Prognostic value of Bcl-2 expression in squamous cell carcinoma of the larynx: A systematic review. Int J Biol Markers 30: e155-e160, 2015.
34. Gioacchini FM, Alicandri-Ciufelli M, Kaleci S, Magliulo G, Presutti L and Re M: The prognostic value of Cyclin D1 expression in head and neck sqamous cell carcinoma. Eur Arch Otorhinolaryngol 273: 801-809, 2016.
35. Gioacchini FM, Alicandri-Ciufelli M, Magliulo G, Rubini C, Presutti L and Re M: The clinical relevance of Ki-67 expression in laryngeal squamous cell carcinoma. Eur Arch Otorhinolaryngol 272: 1569-1576, 2015.
36. Re M, Magliulo G, Salvolini E, Orciani M, Gioacchini FM, Goteri G and Rubini C: Prognostic significance of p53 and KAI-1 expression in patients with Laryngeal squamous cell carcinoma. Anal Quant Cytol Histol 32: 247-253, 2010.
37. Fan CY: Epigenetic alterations in head and neck cancer: Prevalence, clinical significance, and implications. Curr Oncol Rep 6: 152-161, 2004.
38. Marsit CJ, Christensen BC, Houseman EA, Karagas MR, Wrensch MR, Yeh RF, Nelson HH, Wiemels JL, Zheng S, Posner MR, *et al*: Epigenetic profiling reveals etiologically distinct patterns of DNA methylation in head and neck squamous cell carcinoma. Carcinogenesis 30: 416-422, 2009.
39. Worsham MJ, Chen KM, Meduri V, Nygren AO, Errami A, Schouten JP and Benninger MS: Epigenetic events of disease progression in head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 132: 668-677, 2006.
40. Ambros V: The functions of animal microRNAs. Nature 431: 350-355, 2004.

41. Zamore PD and Haley B: Ribo-gnome: The big world of small RNAs. Science 309: 1519-1524, 2005.
42. Schickel R, Boyerinas B, Park SM and Peter ME: MicroRNAs: Key players in the immune system, differentiation, tumorigenesis and cell death. Oncogene 27: 5959-5974, 2008.
43. Cullen BR: Transcription and processing of human microRNA precursors. Mol Cell 16: 861-865, 2004.
44. Avissar M, McClean MD, Kelsey KT and Marsit CJ: MicroRNA expression in head and neck cancer associates with alcohol consumption and survival. Carcinogenesis 30: 2059-2063, 2009.
45. Chen CZ: MicroRNAs as oncogenes and tumor suppressors. N Engl J Med 353: 1768-1771, 2005.
46. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, *et al*: MicroRNA gene expression deregulation in human breast cancer. Cancer Res 65: 7065-7070, 2005.
47. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, *et al*: A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 353: 1793-1801, 2005.
48. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, Zeng YX and Shao JY: MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA 14: 2348-2360, 2008.
49. Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, Yuen ST, Chan TL, Kwong DL, Au GK, *et al*: MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA 299: 425-436, 2008.
50. Chen Y and Stallings RL: Differential patterns of microRNA expression in neuroblastoma are correlated with prognosis, differentiation, and apoptosis. Cancer Res 67: 976-983, 2007.
51. Avissar M, Christensen BC, Kelsey KT and Marsit CJ: MicroRNA expression ratio is predictive of head and neck squamous cell carcinoma. Clin Cancer Res 15: 2850-2855, 2009.
52. Chang SS, Jiang WW, Smith I, Poeta LM, Begum S, Glazer C, Shan S, Westra W, Sidransky D and Califano JA: MicroRNA alterations in head and neck squamous cell carcinoma. Int J Cancer 123: 2791-2797, 2008.
53. Childs G, Fazzari M, Kung G, Kawachi N, Brandwein-Gensler M, McLemore M, Chen Q, Burk RD, Smith RV, Prystowsky MB, *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 174: 736-745, 2009.
54. Ramdas L, Giri U, Ashorn CL, Coombes KR, El-Naggar A, Ang KK and Story MD: miRNA expression profiles in head and neck squamous cell carcinoma and adjacent normal tissue. Head Neck 31: 642-654, 2009.
55. Long XB, Sun GB, Hu S, Liang GT, Wang N, Zhang XH, Cao PP, Zhen HT, Cui YH and Liu Z: Let-7a microRNA functions as a potential tumor suppressor in human laryngeal cancer. Oncol Rep 22: 1189-1195, 2009.
56. Cardesa A, Gale N, Nadal A and Zidar N: Squamus cell carcinoma. In: Head and Neck tumors: Pathology & Genetics. World Health Organization Classification on Tumors (WHO), 2005. p 118-119.
57. Edge SB and Compton CC: The American joint committee on cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 17: 1471-1474, 2010
58. Rampelli V, Ferrari M and Nicolai P: Intestnal-type adenocarcinoma of the sinonasal tract: An update.Curr Opin Otolaryngol Head Neck Surg 26: 115-121, 2018.
59. Nicolai P, Shreiber A, Villaret AB, Lombardi D, Morassi L, Raffetti E, Donato F, Battaglia P, Turri-Zanoni M, Bignami M and Castelnuovo P: Intestinal type adenocarcinoma of the ethmoid: Outcomes of a treatment regimen based on endoscopic surgery with or without radiotherapy. Head Neck 38: E996-E1003, 2016.
60. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
61. Fearon ER and Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 61: 759-767, 1990.
62. Chung DC: The genetic basis of colorectal cancer: Insights into critical pathways of tumorigenesis. Gastroenterology 119: 854-865, 2000.
63. Calvert PM and Frucht H: The genetics of colorectal cancer. Ann Intern Med 137: 603-612, 2002.
64. Licitra L, Suardi S, Bossi P, Locati LD, Mariani L, Quattrone P, Lo Vullo S, Oggionni M, Olmi P, Cantu G, *et al*: Prediction of TP53 status for primary cisplatin, fluorouracil, and leucovorin chemotherapy in ethmoid sinus intestinal-type adenocarcinoma. J Clin Oncol 22: 4901-4906, 2004.
65. Perez-Ordonez B, Huynh NN, Berean KW and Jordan RC: Expression of mismatch repair proteins, beta catenin, and E cadherin in intestinal-type sinonasal adenocarcinoma. J Clin Pathol 57: 1080-1083, 2004.
66. Kennedy MT, Jordan RC, Berean KW and Perez-Ordonez B: Expression pattern of CK7, CK20, CDX-2, and villin in intestinal-type sinonasal adenocarcinoma. J Clin Pathol 57: 932-937, 2004.
67. Esquela-Kerscher A and Slack FJ: Oncomirs-microRNAs with a role in cancer. Nat Rev Cancer 6: 259-269, 2006.
68. Tu HF, Lin SC and Chang KW: MicroRNA aberrances in head and neck cancer: Pathogenetic and clinical significance. Curr Opin Otolaryngol Head Neck Surg 21: 104-111, 2013.
69. Lee YS and Dutta A: MicroRNAs: Small but potent oncogenes or tumor suppressors. Curr Opin Investig Drugs 7: 560-564, 2006.
70. Price C and Chen J: MicroRNAs in cancer biology and therapy: Current status and perspectives. Genes Dis 1: 53-63, 2014.
71. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, *et al*: Reduced expression of the let-7 microTNAs in human lung cancer in association with shortened postoperative survival. Cancer Res 64: 3753-3756, 2004.
72. He X, Duan C, Chen J, Ou-Yang X, Zhang Z, Li C and Peng H: Let-7a elevates p21(WAF1) levels by targeting of NIRF and suppresses the growth of A549 lung cancer cells. FEBS Lett 583: 3501-3507, 2009.
73. Akao Y, Nakagawa Y and Naoe T: Let-7 microRNA functions as a potential growth suppressor in human colon cancer cell. Biol Pharm Bull 29: 903-906, 2006.
74. Colamaio M, Calì G, Sarnataro D, Borbone E, Pallante P, Decaussin-Petrucci M, Nitsch L, Croce CM, Battista S and Fusco A: Let-7a down-regulation plays a role in thyroid neoplasias of follicular histotype affecting cell adhesion and migration through its ability to target the FXYD5 (Dysadherin) gene. J Clin Endocrinol Matab 97: E2168-E2178, 2012.
75. Liu Y, Yin B, Zhang C, Zhou L and Fan J: Hsa-let-7a functionsas a tumor suppressor in renal cell carcinoma cell lines by targetingc-myc. Biochem Biophys Res Commun 417: 371-375, 2012.
76. Yang Q, Jie Z, Cao H, Greenlee AR, Yang C, Zou F and Jiang Y: Low-level expression of let-7a in gastric cancer and its involvement in tumorigenesis by targeting RAB40C. Carcinogenesis 32: 713-722, 2011.
77. De Vito C, Riggi N, Suvà ML, Janiszewska M, Horlbeck J, Baumer K, Provero P and Stamenkovic I: Let-7a is a direct EWS-FLI-1 target implicated in Ewing's sarcoma development. PLoS One 6: e23592, 2011.