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Hypoxia reduces the E-cadherin expression and increases OSCC cell migration regardless of the E-cadherin methylation profile

Patrícia Luciana Batista Domingos[a,1], Marcela Gonçalves Souza[a,1], Talita Antunes Guimarães[a], Eliane Sobrinho Santos[a], Lucyana Conceição Farias[a], Carlos Alberto de Carvalho Fraga[a], Kimberly Marie Jones[b], Sérgio Henrique Souza Santos[c], Alfredo Maurício Batista de Paula[a], André Luiz Sena Guimarães[a,*]

[a] Department of Dentistry, Universidade Estadual de Montes Claros, Montes Claros, Minas Gerais, Brazil
[b] Dean of Research, Associação Educativa do Brasil (SOEBRAS), Montes Claros, Montes Claros, Minas Gerais, Brazil
[c] Institute of Agricultural Sciences, Food Engineering College, Universidade Federal de Minas Gerais (UFMG), Montes Claros, Minas Gerais, Brazil

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ABSTRACT

Objective: The purpose of the current study is to investigate the association between E-cadherin methylation status, hypoxia and OSCC.
Methods: HaCat and SCC9 cell lines were submitted to hypoxic treatment, followed by methylation profile analysis (MS-PCR) and analysis of the expression of mRNA gene E-cadherin (RT-PCR). Study group samples comprise individuals affected by potentially malignant lesions Potential Malignant Oral Lesion (PMOL, n = 18) and oral squamous cell carcinoma (OSCC, n = 28). The control group oral mucosa (OM, n = 15) of patients with an oral mucocele. Cell migration ability was evaluated a scratch wound assay in SCC9 and HaCat cell lines
Results: E-cadherin mRNA expression in the cell lines SCC9 and HaCat was significantly reduced under hypoxia, regardless of the methylation profile, when compared to the control group. No differences in methylation profile of the E-cadherin were observed among the groups OM, PMOL and OSCC. HaCat and SCC9 presented increases in cell migration rates under hypoxia.
Conclusion: The current study demonstrates that hypoxia reduces E-cadherin expression and increase cell migration, regardless of the methylation profile. Additionally, no differences in E-cadherin methylation patterns were observed among OM, PMOL and OSCC.

1. Introduction

Oral cancer is a significant public health problem. Additionally, consequences because oral cancer treatment often produces mutilation and even death threat worldwide [1,2]. Oral squamous cell carcinoma (OSCC) is the most common malignant neoplasm that occurs in the mouth [14] However, survival of patients with OSCC has not significantly improved [15]. The hypoxic environment promotes OSCC development. Hypoxia also contributes to OSCC worse prognosis [1,2,14,15]. Moreover, hypoxic environment increases OSCC cell invasion [19]. Interestingly enough, the changes in the cell-extracellular matrix (ECM) composition also induce tumor invasiveness [33]. The junctional complex is a system of membrane proteins that controls cell to cell adhesion. The junctional complex is responsible for the maintenance of the tissue integrity, morphogenesis, cell communication, cell growth, and cell differentiation [32]. Among the molecules involved in the cell adhesion, the cadherins are involved in the formation and maintenance of solid tissues, as well as in epithelial phenotype maintenance [31]. Recent studies suggest that reduced E-cadherin expression could be used to identify lesions with potential risk for malignant transformation [45].

Epigenetic changes constitute an important mechanism that controls the E-cadherin expression [5]. Specifically, hypermethylation in the promoter region has been identified as an important epigenetic event associated with the reduction of E-cadherin

* Corresponding author at: Universidade Estadual de Montes Claros, Hospital Universitário Clemente Faria, Laboratório de Pesquisa em Saúde, 562 Av. Cula Mangabeira Santo Expedito, Montes Claros, Minas Gerais, Brazil.
E-mail address: andreluizguimaraes@gmail.com (A.L.S. Guimarães).
[1] These authors equally contributed.

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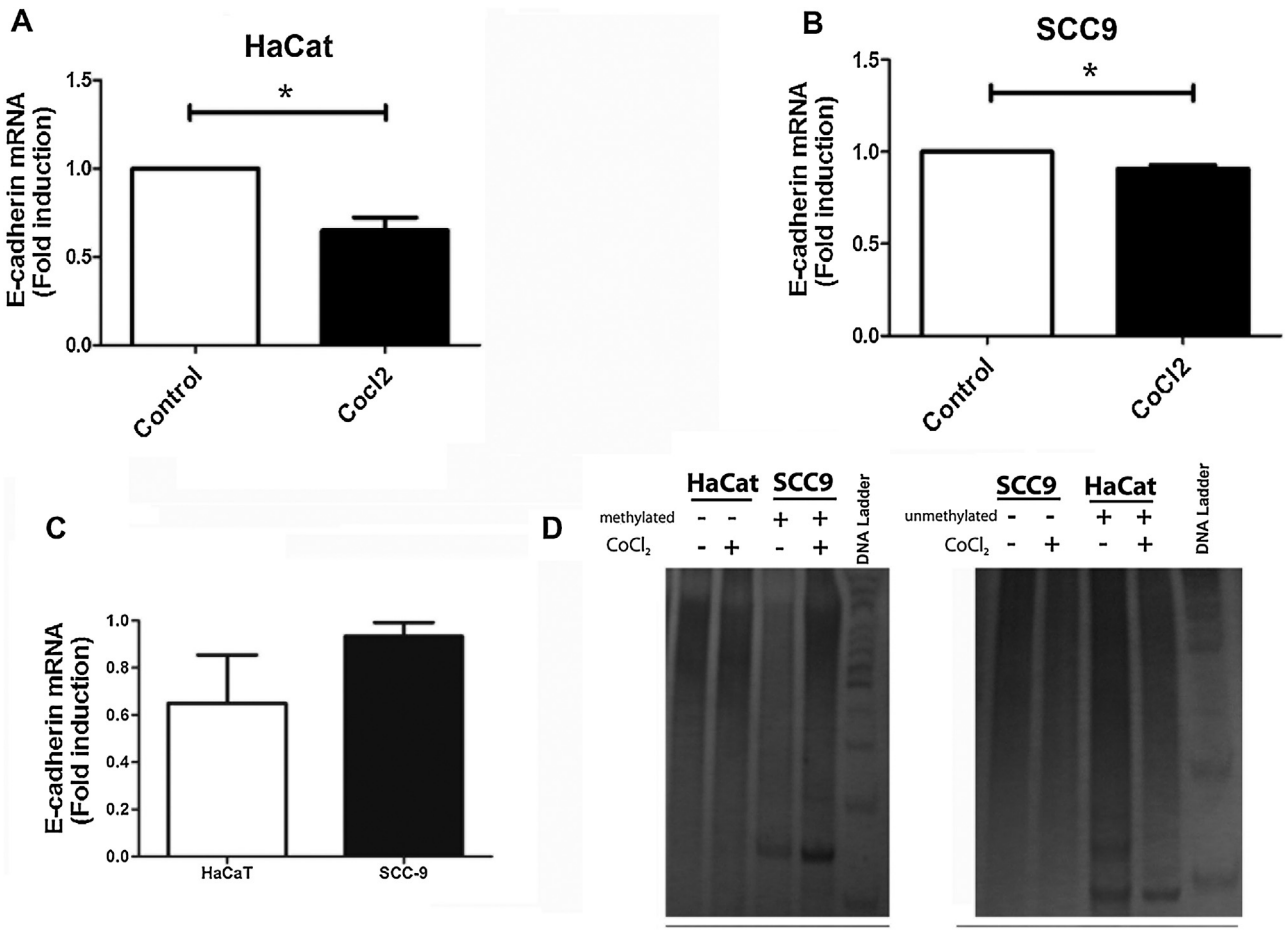

Fig. 1. Expression of E-cadherin mRNA in cell lines under normoxia and hypoxia HaCat (A) and SCC9 (B). Our findings showed a significant decrease of E-cadherin mRNA in cell lines under hypoxic condition compared to the control group. No E-cadherin expression differences were observed between methylated (SCC9) and non-methylated (HaCat) cell lines (C). No changes to E-cadherin methylation profile was observed with hypoxia (D).The statistical analysis was performed using Tukey post test.

expression [9,24]. Decrease in E-cadherin expression or translocation from the plasma membrane to the cytoplasm may influence the development of epithelial carcinogenesis [17]. However, reduction of E-cadherin expression was not associated with TNM [35]. Aberrant methylation of E-cadherin gene might play a role in the progression of OSCC lesions [3] However; literature results concerning the impact of E-cadherin methylation in OSCC development and progression are divergent [41]. Considering that hypoxia is an important factor associated with a reduction of the E-cadherin expression and cell invasion [22], the goal of this study was to investigate the association between E-cadherin methylation status, hypoxia, and OSCC.

2. Methods

2.1. Cell culture

The immortalized cell line of tongue squamous cell carcinoma (SCC9) (ATCC, Manassas, VA, USA) was used. This cell line was cultivated in Dulbecco's modified Eagles medium (DMEM/F12, GIBCO, Billings, MT, USA), with 10% of bovine fetal serum (FBS, GIBCO,Billings, MT, USA), 400 ng/mL hydrocortisone, and an antibiotic/antimycotic solution (Invitrogen, Carlsbad, CA, USA), at 37 °C in a humidified atmosphere of 5% of CO_2. HaCat cells were cultured in DMEM (GIBCO, Billings, MT, USA), supplemented with 10% of FBS at 37 °C with 5% CO_2 in a humidified atmosphere., SCC9 and HaCat cells (1×10^5) were synchronized with 24 h serum starvation to obtain synchronized cultures. All treatments were performed in the absence of FBS.

2.2. Hypoxic treatment

After the cells had been cultivated in a serum-free medium, the cell lines SCC9 and HaCat were divided into two groups: control and submitted to hypoxia treatment. 100 μM of $CoCl_2$, a chemical inductor of hypoxia, was added to the serum-free medium of the treatment group for a 24 h period.

2.3. Wound scratch assay

Cell migration was monitored in a scratch wound assay as described previously [7]. Briefly, a scratch was made with a sterile pipette tip in a confluent cell layer, washed twice in PBS, and then 100 μM of $CoCl_2$ was added to a serum-free medium. Wells were photographed at the beginning of the experiment and after 24 h (SCC9 and HaCat cells). Pictures were obtained with an SC30 camera (Olympus, Center Valley, PA, USA) in an IX81 inverted microscope (Olympus, Center Valley, PA, USA). ImageJ software was used for analysis [38]. The first area (in pixels) was divided by the final cell-free area (in pixels) to calculate the wound healing ratio. The experiment was performed in triplicate.

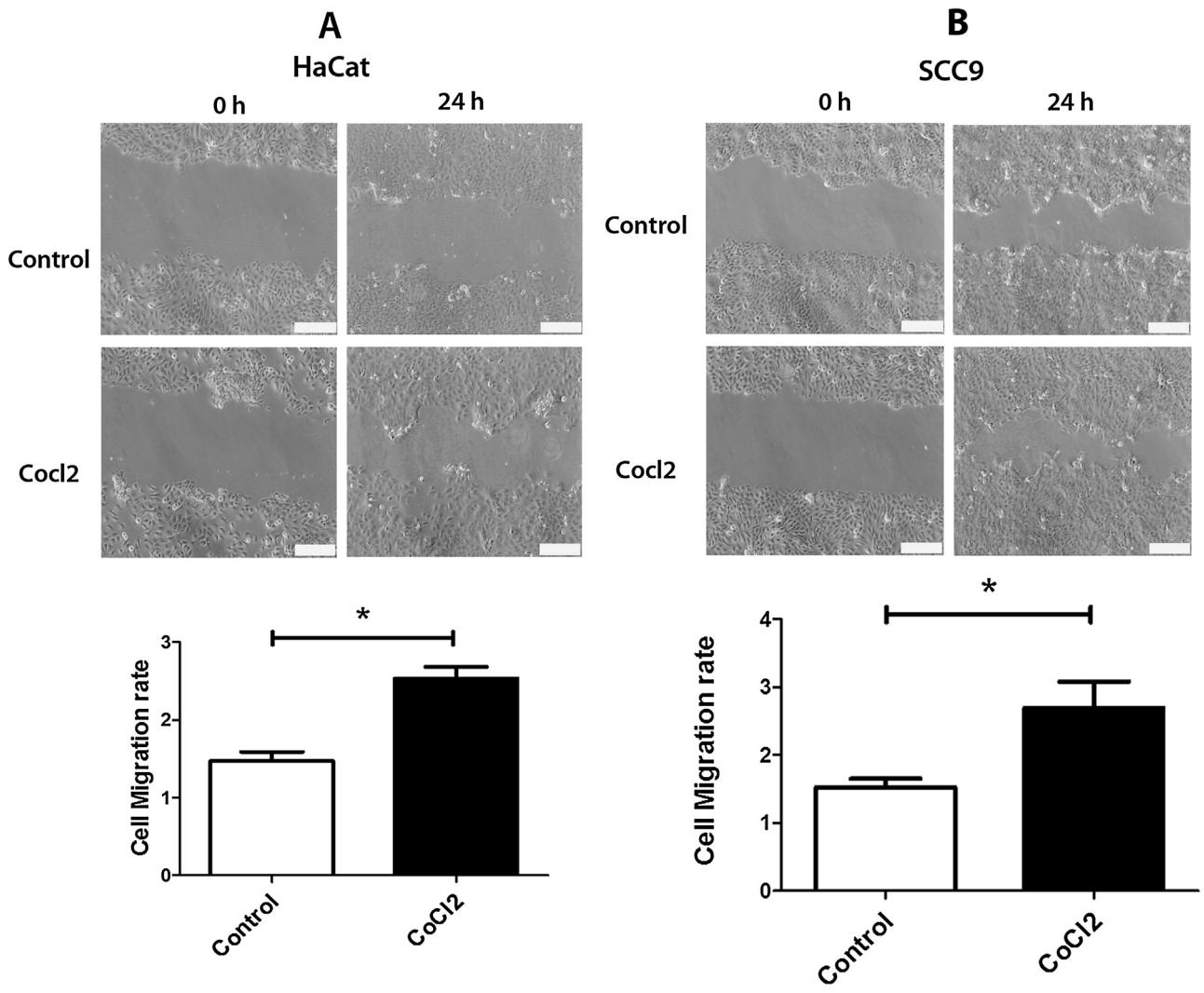

Fig. 2. Effect of $CoCl_2$ on migration in cell line HaCat (A) and SCC9 (C). Our findings showed a significant reduction of the area in the cell line HaCat (B) and SCC9 (D), and consequently, a greater cell migration in the group submitted to hypoxia when compared to the untreated group. The statistical analysis was performed using Tukey post test. The scale bar is 50 μm. * indicates that $p < 0.05$.

2.4. Relative quantitative real-time-PCR

E-cadherin gene expression in the cell lines under study was evaluated by relative quantitative real-time-PCR (qRT-PCR) after carrying out the RNA extraction using Trizol reagent (GIBCO, Billings, MT, USA), according to the manufacturer's protocol. The RNA integrity was evaluated and confirmed through an agarose gel stained with ethidium bromide. 1500 ng of total RNA was converted into complementary DNA (cDNA) using reverse transcriptase enzyme (SuperScript Catalog number: 11904-018, Invitrogen, Carlsbad, CA, USA). For the amplification reactions, 150 ng of cDNA were added to the SYBR® Green PCR Master Mix reagent (Applied Biosystems, Foster City, CA, USA), which contains a fluorescent molecule along with the other necessary reagents required for the amplification. Amplification of cDNA was performed with the primers described in Supplementary Table S1. All the reactions were performed in triplicate. The relative quantification method was adopted to evaluate the transcription levels of the gene under study [25]. This method is based on the comparison of the Cts (Threshold cycle) from each sample with the Ct of an endogenous gene and a calibrating sample with results presented in arbitrary units.

2.5. Analysis of the E-cadherin promoter region methylation

The DNA extraction was performed from cryopreserved samples (−80 °C), using the kit Dneasy Tissue Kit QIAGEN® (Qiagen, Chatsworth, CA), according to the manufacture's protocol. After the extraction, the DNA was treated with sodium bisulfite, which converts the unmethylated cytosines into uracil, as the methylated cytosines have been found to be resistant to this modification [13]. This reaction allows the identification of the gene methylation profile. 200 ng of DNA was amplified by Methylation-Specific PCR (MS-PCR). 0.4 μM of each of the primers (Supplementary Table S2) were used to identify the methylated and unmethylated profiles, in distinct amplifications as described before and in [18]. Other products were employed in MS-PCR, including 0.2 mM of DNTP (Amersham Biosciences, Pittsburgh, PA, USA), 1X PCR buffer, 1 mM magnesium chloride, and 2.5 U of Platinum Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA). Bisulfite-treated unmethylated DNA (Qiagen Inc., Valencia, CA, USA) was used as positive control of the unmethylated reaction. To obtain positive methylated controls, unmethylated DNA (Qiagen Inc., Valencia, CA, USA) was treated with MSssI methylase enzyme (New England Biolabs, Beverly, USA). The PCR products were analyzed by

electrophoresis on 10% acrylamide gel stained with silver, and the methylation profile of each sample was observed.

2.6. Patients

This study involved the analysis of clinical data and biological material from 61 individuals distributed into the following categories: Oral Mucosa (OM, n = 15), Potential Malignant Oral Lesion (PMOL, n = 18) and OSCC (n = 28). As inclusion criteria, all patients should have histopathological diagnoses by the same pathologist. Additionally, only Oral Leukoplakia was used as a PMOL, and oral mucosa was obtained from oral mucoceles surgery.

As criteria for inclusion, all patients had histopathological diagnoses by the same pathologist. Oral mucosa was obtained from oral mucoceles. We observed previously published criteria for PMOL and OSCC diagnoses [26,27]. The health records of these patients were retrieved, and socio-demographic, clinical, and outcome data were extracted. Individuals with injuries and those that did not authorize the use of their data or biological materials were excluded from the study.

2.7. Ethics

Ethical approval for this study was obtained from the relevant Institutional Review Board (CAAE number: 35499414.0.0000.5146). All patients signed a written consent form.

2.8. Statistical analysis

For continuous variables, Kolmogorov-Smirnov and Shapiro-Wilk were used to test the normality of data distribution. Furthermore, data were evaluated using ANOVA and Tukey post hoc test. Categorical data was assessed with Chi-square and Fisherís exact statistical tests. Statistical significance was accepted at $p < 0.05$. All data was analyzed in PASW®, version 18.0 (Windows®). Plots construction were performed in GraphPad Prism software (Version 5.0, GraphPad Software Inc., San Diego, CA, USA).

3. Results

Hypoxia reduces the mRNA E-cadherin expression and increases cell migration, regardless of the methylation profile

Hypoxia increased significantly E-cadherin mRNAs comparison to the control group for the cell lines HaCat and SCC9 (Fig. 1A and B respectively). On the other hand, no differences in E-cadherin was observed between HaCat and SCC9 cells (Fig. 1C). The methylation profile of cells lines HaCat and SCC9 was evaluated by Methylation-Specific-PCR (MS-PCR). The E-cadherin gene presented as methylated in SCC9 cells and as unmethylated in HaCat cells (Fig. 1D). The hypoxic environment did not change the methylation profile of the E-cadherin gene in both cell lines (Fig. 1D).

Invasiveness is an important characteristic of OSCC cells and a target for the development of biomarkers. Scratch wound assay evaluated the impact of the E-cadherin mRNAs levels on cell migration ability. As shown in Fig. 2, hypoxia increased SCC9 and HaCat cell migration.

There are no differences in the E-cadherin between the methylation profiles in OM, PMOL, and OSCC

E-cadherin methylation profiles of OM, PMOL, and OSCC were compared (Table 1) According to our findings, most of the samples presented unmethylated E-cadherin gene [12 (80,0%) of OM, in 12 (66,7%) of PMOL and 21 (75%) of OSCC]. Also, comparison among OM, PMOL, and OSCC showed no significant difference in E-cadherin methylation status ($p > 0.05$, Table 1). Furthermore, our findings did not show a significant association between E-cadherin methylation status and clinicopathological variables (Supplementary Table S3–S5).

Table 1
Distribution of the groups according to the E-cadherin methylation status.

Variables	E-cadherin Methylation Status		p
	Methylated	Non-methylated	
Groups			
Oral mucosa	3 (20.0%)	12 (80.0%)	
Potentially Malignant Oral Lesions	6 (33.3%)	12 (66.7%)	0.754
OSCC	7 (25%)	21 (75%)	
SCC9 cells	100%	0%	
SCC9 cells under hipoxia	100%	0%	NA
HaCaT cells	0%	100%	
HaCaT cells under hipoxia	0%	100%	NA

* Indicates that $p < 0.05$.

4. Discussion

Induction of epithelial-mesenchymal transition mechanisms (EMT) and associated decreases in the expression of E-cadherin gene are essential factors in carcinogenesis [47]. Reduction of E-cadherin expression is considered the central event in the metastatic process since there is a reduction of the cell adhesion that permits the invasion of tumor cells [4]. The maintenance of cell to cell adhesion may deter the migration and invasion of tumor cells as well differentiated cells, favoring the passive migration and invasion of cancer cells [34]. In the current study, hypoxic environment interfered in E-cadherin mRNA levels in SCC9 and HaCat cells. There was a significant reduction of the mRNA expression levels of E-cadherin in the cells under hypoxia in SCC9 and HaCat when compared to the control group. Our data corroborate with previous study wich demonstrate the important role for hypoxia in mediating the effects of E-cadherin expression as well as invasiveness in human ovarian cancer cells [10]. Hypoxic conditions in cancer tissues exert strong selective pressures that favor the survival and proliferation of cancer cells, contributing to the malignant phenotype of tumor aggressiveness [20]. Studies have demonstrated that E-cadherin may influence the process of epithelial-mesenchymal transition (EMT) through the regulation of HIF-1α [37]. HIF induces the expression of a repressor SNAIL that decreases the transcription of E-cadherin [12,29,44]. Consequently, reduction of E-cadherin leads to a disruption of the epithelial organization, contributing to metastatic processes [30].

Different molecular mechanisms could regulate E-cadherin expression in the context of malignant neoplasms [5,9,47]. Epigenetic silencing, specifical hypermethylation of the promoter region, is associated with inactivation of the E-cadherin, contributing to carcinogenesis [5,9,24,47]. The present study demonstrates that hypoxic environments did not change the methylation profile of E-cadherin in cell cultures. Also, no differences in E-cadherin methylation patterns were observed tissues with different oxygen tensions (OM, PMOL and OSCC). Interestingly enough, promoter methylation of INK4A, cytoglobin, E-cadherin, and TMEFF2 did not add prognostic information to histopathological reporting of resection margins in oral cancer [40]. Additionally, no correlation of E-cadherin hypermethylation was detected in OSCC histopathologically negative surgical margins with clinical and prognostic parameters [42]. However, contradictory results are found in the literature [9,36]. Interesting enough in some previous study [9,46] no association between E-cadherin methylation and survival was observed. The methodology to evaluate the effect of methylation might be responsible for divergence in results. In another study

[36], for example, used immunohistochemistry to assess the effect of methylation on the expression of E-cadherin. The immunohistochemistry detection could stain inactive proteins and other technical pitfalls [8,43]. It is important to highlight that E-cadherin gene methylation was also associated with tumors with lowest invasiveness and metastatic potential [11].

The divergence from previous studies [3] might be related to the sample size and differences in tobacco usage between the two populations. It is important to note that other mechanisms can contribute to gene silencing [28].

In general, activation of HIF-1α under hypoxic conditions modulates a variety of processes such as glycolysis and angiogenesis glucose transport [23,39] favoring cell growth proliferation [6,16,21]. The HIF-1α expression is very common in solid tumors, providing a suggested route to decrease E-cadherin expression [12]. Our study indicated that hypoxia increased cell migration.

In conclusion, the current study demonstrates that hypoxia reduces E-cadherin expression and increase cell migration, regardless of the methylation profile. Additionally, no differences in E-cadherin methylation patterns were observed among OM, PMOL and OSCC.

Conflicts of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prp.2017.02.003.

References

[1] C.M. Almeida, S.F. Jesus, O. Poswar Fde, E.S. Gomes, C.A. Fraga, L.C. Farias, S.H. Santos, J.D. Feltenberger, A.M. Paula, A.L. Guimaraes, Increasing demonstration of angiogenic markers in skin neoplastic lesions, Pathol. Res. Pract. 212 (2016) 101–105.
[2] L.R. Alves, C.A. Fraga, M.V. Oliveira, A.A. Sousa, A.S. Jorge, L. Marques-Silva, S.H. Santos, K.M. Jones, A.M. de Paula, A.L. Guimaraes, High HIF-1alpha expression genotypes increase odds ratio of oral cancer, Head Neck Oncol. 4 (2012) 87.
[3] G.S. Asokan, S. Jeelani, N. Gnanasundaram, Promoter hypermethylation profile of tumour suppressor genes in oral leukoplakia and oral squamous cell carcinoma. Journal of clinical and diagnostic research, JCDR 8 (2014) ZC09–12.
[4] I.R. Beavon, The E-cadherin-catenin complex in tumour metastasis: structure function and regulation, Eur. J. Cancer 36 (2000) 1607–1620.
[5] G. Berx, F. van Roy, Involvement of members of the cadherin superfamily in cancer, Cold Spring Harbor Perspect. Biol. 1 (2009) a003129.
[6] C. Brahimi-Horn, N. Mazure, J. Pouyssegur, Signalling via the hypoxia-inducible factor-1alpha requires multiple posttranslational modifications, Cell. Signal. 17 (2005) 1–9.
[7] I.J. Brusevold, M. Aasrum, M. Bryne, T. Christoffersen, Migration induced by epidermal and hepatocyte growth factors in oral squamous carcinoma cells in vitro: role of MEK/ERK, p38 and PI-3 kinase/Akt, J. Oral Pathol. Med. 41 (547) (2012).
[8] G. Bussolati, E. Leonardo, Technical pitfalls potentially affecting diagnoses in immunohistochemistry, J. Clin. Pathol. 61 (2008) 1184–1192.
[9] H.W. Chang, V. Chow, K.Y. Lam, W.I. Wei, A. Yuen, Loss of E-cadherin expression resulting from promoter hypermethylation in oral tongue carcinoma and its prognostic significance, Cancer 94 (2002) 386–392.
[10] J.C. Cheng, C. Klausen, P.C. Leung, Hypoxia-inducible factor 1 alpha mediates epidermal growth factor-induced down-regulation of E-cadherin expression and cell invasion in human ovarian cancer cells, Cancer Lett. 329 (2013) 197–206.
[11] R.V. de Moraes, D.T. Oliveira, G. Landman, F. de Carvalho, O. Caballero, S. Nonogaki, I. Nishimoto, L.P. Kowalski, E-cadherin abnormalities resulting from CPG methylation promoter in metastatic and nonmetastatic oral cancer, Head Neck 30 (2008) 85–92.
[12] M.A. Esteban, M.G. Tran, S.K. Harten, P. Hill, M.C. Castellanos, A. Chandra, R. Raval, T.S. O'Brien, P.H. Maxwell, Regulation of E-cadherin expression by VHL and hypoxia-inducible factor, Cancer Res. 66 (2006) 3567–3575.
[13] L.C. Farias, C.A. Fraga, M.V. De Oliveira, T.F. Silva, L. Marques-Silva, P.R. Moreira, A.M. De-Paula, R.S. Gomez, A.L. Guimaraes, Effect of age on the association between p16CDKN2A methylation and DNMT3B polymorphism in head and neck carcinoma and patient survival, Int. J. Oncol. 37 (2010) 167–176.
[14] C.A. Fraga, M.V. de Oliveira, E.S. de Oliveira, L.O. Barros, F.B. Santos, R.S. Gomez, A.M. De-Paula, A.L. Guimaraes, A high HIF-1alpha expression genotype is associated with poor prognosis of upper aerodigestive tract carcinoma patients, Oral Oncol. 48 (2012) 130–135.
[15] C.A. Fraga, A.A. Sousa, G.T. Correa, A.S. Jorge, S.F. Jesus, K.M. Jones, C.M. Cardoso, L.C. Farias, R.S. Gomez, A.M. de Paula, A.L. Guimaraes, High hypoxia-inducible factor-1alpha expression genotype associated with Eastern Cooperative Oncology Group performance in head and neck squamous cell carcinoma, Head Neck Oncol. 4 (2012) 77.
[16] A. Galanis, A. Pappa, A. Giannakakis, E. Lanitis, D. Dangaj, R. Sandaltzopoulos, Reactive oxygen species and HIF-1 signalling in cancer, Cancer Lett. 266 (2008) 12–20.
[17] N.A. Gloushankova, Changes in regulation of cell-cell adhesion during tumor transformation, Biochemistry Biokhimiia 73 (2008) 742–750.
[18] D. Goldenberg, S. Harden, B.G. Masayesva, P. Ha, N. Benoit, W.H. Westra, W.M. Koch, D. Sidransky, J.A. Califano, Intraoperative molecular margin analysis in head and neck cancer, Arch. Otolaryngol. Head Neck Surg. 130 (2004) 39–44.
[19] T.A. Guimaraes, L.C. Farias, C.A. Fraga, J.D. Feltenberger, G.A. Melo, R.D. Coletta, S.H. Souza Santos, A.M. de Paula, A.L. Guimaraes, Evaluation of the antineoplastic activity of gallic acid in oral squamous cell carcinoma under hypoxic conditions, Anticancer Drugs 27 (2016) 407–416.
[20] J.J. Haddad, H.L. Harb, Cytokines and the regulation of hypoxia-inducible factor (HIF)-1alpha, Int. Immunopharmacol. 5 (2005) 461–483.
[21] M. Hockel, P. Vaupel, Tumor hypoxia: definitions and current clinical biologic, and molecular aspects, J. Natl. Cancer Inst. 93 (2001) 266–276.
[22] T. Imai, A. Horiuchi, C. Wang, K. Oka, S. Ohira, T. Nikaido, I. Konishi, Hypoxia attenuates the expression of E-cadherin via up-regulation of SNAIL in ovarian carcinoma cells, Am. J. Pathol. 163 (2003) 1437–1447.
[23] W.G. Kaelin Jr., Molecular basis of the VHL hereditary cancer syndrome, Nat. Rev. Cancer 2 (2002) 673–682.
[24] T. Kanazawa, T. Watanabe, S. Kazama, T. Tada, S. Koketsu, H. Nagawa, Poorly differentiated adenocarcinoma and mucinous carcinoma of the colon and rectum show higher rates of loss of heterozygosity and loss of E-cadherin expression due to methylation of promoter region, Int. J. Cancer 102 (2002) 225–229.
[25] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408.
[26] R. Macey, T. Walsh, P. Brocklehurst, A.R. Kerr, J.L. Liu, M.W. Lingen, G.R. Ogden, S. Warnakulasuriya, C. Scully, Diagnostic tests for oral cancer and potentially malignant disorders in patients presenting with clinically evident lesions, Cochrane Database Syst. Rev. 5 (2015) CD010276.
[27] H. Mortazavi, M. Baharvand, M. Mehdipour, Oral potentially malignant disorders: an overview of more than 20 entities, J. Dental Res. Dent. Clin. Dent. Prospects 8 (2014) 6–14.
[28] S.J. Nass, J.G. Herman, E. Gabrielson, P.W. Iversen, F.F. Parl, N.E. Davidson, J.R. Graff, Aberrant methylation of the estrogen receptor and E-cadherin 5' CpG islands increases with malignant progression in human breast cancer, Cancer Res. 60 (2000) 4346–4348.
[29] M.A. Nieto, The snail superfamily of zinc-finger transcription factors, Nat. Rev. Mol. Cell Biol. 3 (2002) 155–166.
[30] P. Nistico, M.J. Bissell, D.C. Radisky, Epithelial-mesenchymal transition: general principles and pathological relevance with special emphasis on the role of matrix metalloproteinases, Cold Spring Harbor Perspect. Biol. 4 (2012).
[31] M. Ozawa, W. Kobayashi, Cadherin cytoplasmic domains inhibit the cell surface localization of endogenous E-cadherin, blocking desmosome and tight junction formation and inducing cell dissociation, PLoS One 9 (2014) e105313.
[32] Y.C. Rajwar, N. Jain, G. Bhatia, N. Sikka, B. Garg, E. Walia, Expression and significance of cadherins and its subtypes in development and progression of oral cancers: a review, J. Clin. Diagn. Res 9 (2015), ZE05-07.
[33] O. Ramos Gde, L. Bernardi, I. Lauxen, M. Sant'Ana Filho, A.R. Horwitz, M.L. Lamers, Fibronectin modulates cell adhesion and signaling to promote single cell migration of highly invasive oral squamous cell carcinoma, PLoS One 11 (2016) e0151338.
[34] F.J. Rodriguez, L.J. Lewis-Tuffin, P.Z. Anastasiadis, E-cadherin's dark side: possible role in tumor progression, Biochim. Biophys. Acta 1826 (2012) 23–31.
[35] P. Rosado, P. Lequerica-Fernandez, S. Fernandez, E. Allonca, L. Villallain, J.C. de Vicente, E-cadherin and beta-catenin expression in well-differentiated and moderately-differentiated oral squamous cell carcinoma: relations with clinical variables, Br. J. Oral Maxillofacial Surg. 51 (2013) 149–156.
[36] Y. Saito, H. Takazawa, K. Uzawa, H. Tanzawa, K. Sato, Reduced expression of E-cadherin in oral squamous cell carcinoma: relationship with DNA methylation of 5' CpG island, Int. J. Oncol. 12 (1998) 293–298.

[37] R. Schietke, C. Warnecke, I. Wacker, J. Schodel, D.R. Mole, V. Campean, K. Amann, M. Goppelt-Struebe, J. Behrens, K.U. Eckardt, M.S. Wiesener, The lysyl oxidases LOX and LOXL2 are necessary and sufficient to repress E-cadherin in hypoxia: insights into cellular transformation processes mediated by HIF-1, J. Biol. Chem. 285 (2010) 6658–6669.
[38] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis, Nat. Methods 9 (2012) 671–675.
[39] G.L. Semenza, Targeting HIF-1 for cancer therapy, Nat. Rev. Cancer 3 (2003) 721–732.
[40] R.J. Shaw, A.J. Hobkirk, G. Nikolaidis, J.A. Woolgar, A. Triantafyllou, J.S. Brown, T. Liloglou, J.M. Risk, Molecular staging of surgical margins in oral squamous cell carcinoma using promoter methylation of p16(INK4A), cytoglobin E-cadherin, and TMEFF2, Ann. Surg. Oncol. 20 (2013) 2796–2802.
[41] R.J. Shaw, T. Liloglou, S.N. Rogers, J.S. Brown, E.D. Vaughan, D. Lowe, J.K. Field, J.M. Risk, Promoter methylation of P16, RARbeta E-cadherin, cyclin A1 and cytoglobin in oral cancer: quantitative evaluation using pyrosequencing, Br. J. Cancer 94 (2006) 561–568.
[42] G. Supic, R. Kozomara, N. Jovic, K. Zeljic, Z. Magic, Prognostic significance of tumor-related genes hypermethylation detected in cancer-free surgical margins of oral squamous cell carcinomas, Oral Oncol. 47 (2011) 702–708.
[43] M. Vered, I. Allon, A. Buchner, D. Dayan, E-cadherin in oral SCC: an analysis of the confusing literature and new insights related to its immunohistochemical expression, Histol. Histopathol. 27 (2012) 141–150.
[44] A.E. Vernon, C. LaBonne, Tumor metastasis: a new twist on epithelial-mesenchymal transitions, Curr. Biol. 14 (2004) R719–721.
[45] S.V. von Zeidler, T. de Souza Botelho, E.F. Mendonca, A.C. Batista, E-cadherin as a potential biomarker of malignant transformation in oral leukoplakia: a retrospective cohort study, BMC Cancer 14 (2014) 972.
[46] K.T. Yeh, M.C. Shih, T.H. Lin, J.C. Chen, J.Y. Chang, C.F. Kao, K.L. Lin, J.G. Chang, The correlation between CpG methylation on promoter and protein expression of E-cadherin in oral squamous cell carcinoma, Anticancer Res. 22 (2002) 3971–3975.
[47] K. Yoshiura, Y. Kanai, A. Ochiai, Y. Shimoyama, T. Sugimura, S. Hirohashi, Silencing of the E-cadherin invasion-suppressor gene by CpG methylation in human carcinomas, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 7416–7419.