Collagen type XI α1 facilitates head and neck squamous cell cancer growth and invasion

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Background: Although it is well established that the extracellular matrix affects tumour progression, not much is known about the various components and their effect on head and neck squamous cell carcinoma (HNSCC) progression. Levels of collagen type XI α1 (colXIα1), a minor fibrillar collagen, have been shown to be increased in tumour compared with normal tissue in several cancers, including colorectal, breast, and non-small cell lung cancer. Currently, the functional significance of colXIα1 is not understood.

Methods: We examined the expression levels of colXIα1 mRNA and elucidated the functional role of colXIα1 in HNSCC. Cell proliferation, invasion, and migration were examined with and without colXIα1 knockdown with siRNA in HNSCC.

Results: Our data demonstrate that colXIα1 expression is increased in tumour samples compared with levels in normal adjacent tissue in 16/23 HNSCC patients. In addition, colα11 is increased in HNSCC cell lines compared with normal immortalised epithelial cells and is increased in tumour-derived fibroblasts compared with normal fibroblasts. Using an siRNA approach, we demonstrate that colXIα1 contributes to proliferation, migration, and invasion of HNSCC.

Conclusion: Our cumulative findings suggest that colXIα1 contributes to HNSCC tumorigenesis and may serve as a potential therapeutic target.

Worldwide, head and neck cancer is the 6th leading cause of cancer mortality, with 350 000 deaths per year due to cancers of the oral cavity, pharynx, and larynx (Ferlay et al, 2008). Over 630 000 new cases of head and neck cancer are diagnosed annually, of which 90% are squamous cell carcinoma (HNSCC). Treatment with standard modalities, including chemotherapy, radiation, and surgery, is associated with a 5-year survival rate of only 50%, emphasising the need for newer, more effective approaches (Cognetti et al, 2008). Although cumulative evidence supports the epidermal growth factor receptor (EGFR) as a therapeutic target in HNSCC, where increased expression correlates with worse prognosis (Rubin Grandis et al, 1998), treatment with the EGFR-targeted monoclonal antibody cetuximab has not been shown to prevent metastasis (Bonner et al, 2006).

In a previous study, we used a 12 000 gene oligonucleotide microarray of paired tumour and normal tissue from nine HNSCC patients to determine differential gene expression in order to identify potential therapeutic targets (Sok et al, 2003). Gene expression changes were found in 227 genes, including collagen type XI subunit z1 (colXIz1), which was found in all nine tumours with virtually no expression in normal tissue.

Type XI collagen, a minor fibrillar collagen, is a heterotrimer composed of z1, z2, and z3 chains that co-polymerise with type II and IX collagen to form the cartilage (Spranger, 1998). The z1 subunit produced by non-cartilaginous tissues, such as bone, skin, and arterial smooth muscle cells, is located on chromosome 1 and regulates the promoter of the major collagen type V (COL5a2) (Yoshioka et al, 1995). Although type XI collagenopathies at birth

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3049
are known to produce phenotypes that include facial anomalies, cleft palate, and hearing defects (Spranger, 1998), relatively little is known about the function of type XI collagen in the adult.

A 2001 study of colorectal tumours first identified an association between colXIX1 and cancer (Fischer et al., 2001b). ColXIX1 mRNA expression was found in 20 of 24 colorectal tumours, whereas no apparent expression was found in the 4 normal tissue samples. In addition, COL5a2, which is normally expressed in human fetal gut and not expressed in the normal adult colon tissue, was correlated with the expression of colXIX1 in the colorectal tumours. Recent studies in several other areas of cancer research, including gastric (Vecchi et al., 2007; Zhao et al., 2009), pancreatic (Badea et al., 2008), breast (Ellsworth et al., 2009), and non-small lung cancer (Chong et al., 2006), have also demonstrated increased levels of colXIX1 in tumour tissue compared with less-diseased or normal tissue. In a multi-cancer computational analysis of four data sets of ovarian and colorectal cancer, the authors concluded that colXIX1 is a higher expression of colXIX1 had metastasised to the lymph nodes demonstrated over seven-fold overexpression is an independent predictor of survival (Kim et al., 2010).

Our initial observation of increased expression of colXIX1 in HNSCC was corroborated by another group whose findings also implicated colXIX1 in the pathogenesis of HNSCC metastasis to the lymph nodes (Schmalbach et al., 2004). Primary tumours that had metastasised to the lymph nodes demonstrated over seven-fold higher expression of colXIX1 than tumours that had not metastasised and over 25-fold higher expression of colXIX1 than levels in normal oral cavity mucosa samples. Thus far, the precise role of colXIX1 in proliferation and invasion remains incompletely understood, and few functional data have been reported.

In the present study, we examined colXIX1 expression in a panel of HNSCC cell lines and a cohort of paired tumour/normal tissue samples derived from HNSCC patients. We then used an siRNA approach to determine the role of colXIX1 in cellular proliferation, migration, and invasion.

**MATERIALS AND METHODS**

**Cell lines, tumours, and reagents.** Human HNSCC cell lines included UMSCC-1 (Krause et al., 1981), UMSCC-10A (Grenman et al., 1991), UMSCC-10B (Somers et al., 1992), UMSCC-22A (Lansford et al., 1999), and UMSCC-22B (Grenman et al., 1991), which were kind gifts from Dr. Thomas Carey (University of Michigan); UPCI-15B (Snyderman et al., 1994) from Dr. Teresa Whiteside (University of Pittsburgh); 1483 (Sacks et al., 1988) from Dr. Peter Sacks (New York University); 686LN (Sturgis et al., 1994) from Dr. Georgia Chen (Emory University); and FaDu (Rangan, 1972) from Dr. Jeffrey Myers (The University of Texas MD Anderson Cancer Center). The Het1A (Stoner et al., 1991) cell line, derived from mucosal epithelial cells, was obtained from American Type Culture Collection (Manassas, VA, USA). All cell lines were validated through genotyping with an AmpF/STR Identifier PCR Amplification Kit (Applied Biosystems, Carlsbad, CA, USA).

UMSCC-1 and FaDu cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen, Carlsbad, CA, USA) with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen) and 0.4 µl ml⁻¹ hydrocortisone. 686LN cells were maintained in Dulbecco’s modified Eagle’s medium/F12 (1:1) (Invitrogen) and 10% FBS. Het1A cells were maintained in Airway Epithelial Cell Growth Media (PromoCell, Heidelberg, Germany) with a supplement provided by the company. All other cell lines including cancer-associated fibroblasts (CAFs), and normal fibroblasts were maintained in DMEM with 10% FBS. All cells were incubated at 37 °C in the presence of 5% CO2. Fibroblast cultures were grown from HNSCC tissue or from uvulopalatoplasty or tonsillectomy from cancer-free subjects. Written consent was collected from patients, using a University of Pittsburgh, Institutional Review Board approved protocol. In brief, tissue pieces were minced and placed in 10% serum containing media. Fibroblast cultures free of epithelial cells were used in these studies.

Primary HNSCC tumour samples, as well as normal mucosal samples adjacent to the tumour site, were obtained under an institutional review board-approved protocol from 23 HNSCC patients undergoing surgical excision with curative intent (Table 1). Signed informed consent was obtained from each subject. Human papilloma virus (HPV) status was not determined for majority of the patients (58%). All evaluated patients were HPV negative.

**Table 1. Summary of HNSCC patient and tumour characteristics**

| Characteristic | HNSCC cases (N = 23) |
|---------------|---------------------|
| Age, years    |                     |
| Median (range)| 63 (32–88)          |
| Sex, N (%)    |                     |
| Men           | 17 (73.9)           |
| Women         | 6 (26.1)            |
| Smoking, N (%)|                     |
| Never         | 2 (8.7)             |
| Former Smoker | 8 (34.8)            |
| Active Smoker | 13 (56.5)           |
| Alcohol, N (%)|                     |
| Never         | 8 (34.8)            |
| Ever          | 15 (65.2)           |
| Cancer site, N (%) |               |
| Oral cavity   | 16 (79.6)           |
| Pharynx       | 1 (4.3)             |
| Larynx        | 4 (17.4)            |
| Other         | 2 (8.7)             |
| Cancer type, N (%) |              |
| Primary       | 20 (87.0)           |
| Recurrence    | 3 (13.0)            |
| Tumour path stage, N (%) |           |
| T1-T2         | 8 (34.8)            |
| T3-T4         | 12 (52.2)           |
| Unstaged recurrent tumour | 3 (13.0) |
| Nodal path stage, N (%) |          |
| N0-N1         | 12 (52.2)           |
| N2-N4         | 8 (34.8)            |
| Unstaged recurrent tumour | 3 (13.0) |
| Perineural invasion, N (%) |            |
| Yes           | 14 (60.9)           |
| No            | 7 (30.4)            |
| Not evaluated | 2 (8.7)             |
| HPV, N (%)    |                     |
| Positive      | 0 (0.0)             |
| Negative      | 9 (39.1)            |
| Not evaluated | 14 (60.9)           |
RT–PCR analysis for colXIa1. Total RNA was isolated from a 30 mg of snap-frozen tumour tissue as described previously using an RNeasy Mini Kit (Qiagen). Total RNA was isolated from 5 × 10^6 cells from each of the nine representative HNSCC cell lines (UMSCC-1, UMSCC-10A, UMSCC-10B, UMSCC-22A, UMSCC-22B, UPCI-15B, 1483, 686LN, and FaDu), normal mucosal epithelial cell line Het1A (5 × 10^6 cells), and fibroblasts, using an RNeasy Mini kit (Qiagen). One nanogram of total RNA was added to a final reaction volume of 25 μl. To detect colXIa1, standard RT–PCR was done using a One-Step RT–PCR kit (Qiagen) with primers (5′-GGATCACAATGAGGAGATGCTCTATG-3′ and antisense sequence as 5′-CTAATGTACCTGTATATGGCAGTGTTG-3′) as described above to amplify a 345-bp fragment. Actin primers were used as previously described (Sok et al., 2006). Primers were diluted to a final concentration of 0.6 μM. All other reagents were used according to the manufacturer’s protocol. Reverse transcription was performed at 50 °C for 30 min, followed by enzyme inactivation and PCR hot start at 95 °C for 15 min. Denaturation, annealing, and extension were performed at 94 °C, 55 °C, and 72 °C, respectively, for 1 min each for a total of 35 cycles. The reaction was completed with an extension period at 72 °C for 10 min. PCR products were visualised on a 1% agarose gel containing ethidium bromide. Expression levels of colXIa1 and actin in the paired tumour and normal epithelial samples were compared using ImageJ densitometry software.

Total RNA was isolated from 5 × 10^6 cells from each of the nine representative HNSCC cell lines (UMSCC-1, UMSCC-10A, UMSCC-10B, UMSCC-22A, UMSCC-22B, UPCI-15B, 1483, 686LN, and FaDu), normal mucosal epithelial cell line Het1A (5 × 10^6 cells), and fibroblasts, using an RNeasy Mini kit (Qiagen). RT–PCR to detect colXIa1 was performed as described above.

ColXIa1 siRNA transfection. Four different siRNA duplexes (A–D) targeted against human colXIa1 siRNA were generated according to the Assays-on-Demand system (Dharmacon, Lafayette, CO, USA): duplex (A) GCAAAUUGGUGUUGAGGUAU, duplex (B) GAAACGUGGGUCAGCAGGUAU, duplex (C) AAAGGGGACUCCGGGUAU, and duplex (D) UCUGGGAGAUGGAGAUAU. Pooled siRNA-colXIa1 was formed by combining all four duplexes. One day before transfection, UPCI-15B cells were plated at 0.5 × 10^5 cells into 24-well plates in order to attain 80–90% confluency at the time of transfection. Twenty-picomole siRNA was transfected in cells with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) in Opti-MEM media.

In vitro growth inhibition by colXIa1 siRNA. To examine the effects of colXIa1 on growth kinetics, UMSCC1 cells were treated with colXIa1 siRNA B or control non-targeting siRNA. Around 42 h post transfection, the cells were trypsinised and plated in xCelligence E-plates Roche (Indianapolis, IN, USA) as previously described (Hickok et al., 2011). In brief, after baseline measurements, 2000 cells were plated in 200 μl DMEM +10% FBS in quadruplicates into 16-well E-plates. Cell index (difference between the impedance at a given time point and the background, divided average resistance across the plate in media only using 10 kHz frequency of current) measurements were taken every 15 min. The growth rates of the cells were assessed in real-time over 40 h for average resistance across the plate in media only using 10 kHz frequency of current) measurements were taken every 15 min. The growth rates of the cells were assessed in real-time over 40 h. To determine if the transformed epithelial cells were the likely source of colXIa1, we compared colXIa1 mRNA levels in tumour tissue compared with levels in paired adjacent normal tissue in 23 HNSCC patients using RT–PCR. In 16 of 23 pairs, colXIa1 expression was significantly higher in the tumour compared with normal tissue (P = 0.0006) with a median fold increase of 6.63 (range 1.8–169), suggesting that increased colXIa1 represents a later event in HNSCC carcinogenesis (Figure 1). In the absence of specific antibodies of sufficiently high quality, we could not validate these expression results at the protein level. However, our results confirm earlier studies demonstrating increased expression of colXIa1 in HNSCC tumours.

Increased expression of colXIa1 in HNSCC tumours compared with levels in adjacent normal mucosa. Previous cDNA microarray analysis demonstrated that, although colXIa1 was present in tumour tissue from all nine HNSCC tumours examined, it was virtually undetectable in corresponding normal adjacent tissue (Sok et al., 2003). The high incidence of synchronous and metachronous tumours of the upper aerodigestive tract that characterises HNSCC carcinogenesis suggests that molecular changes that are found in both the tumour and adjacent normal mucosa may represent relatively early genetic changes (Bedi et al., 1996; Scholes et al., 1998). We sought to determine whether colXIa1 mRNA levels in tumour tissue compared with levels in paired adjacent normal tissue in 23 HNSCC patients using RT–PCR. In 16 of 23 pairs, colXIa1 expression was significantly higher in the tumour compared with normal tissue (P = 0.0006) with a median fold increase of 6.63 (range 1.8–169), suggesting that increased colXIa1 represents a later event in HNSCC carcinogenesis (Figure 1). In the absence of specific antibodies of sufficiently high quality, we could not validate these expression results at the protein level. However, our results confirm earlier studies demonstrating increased expression of colXIa1 in HNSCC tumours.

Increased expression of colXIa1 in HNSCC cell lines compared with normal immortalised mucosal epithelial cells. Tumour lysates represent both epithelial and stromal cells in the tissue microenvironment. There are no reports of colXIa1 expression in cancer cell lines compared with corresponding normal cells. To determine if the transformed epithelial cells were the likely source of colXIa1, we compared colXIa1 mRNA levels in nine genotypically validated, representative HNSCC cell lines (UMSCC-1, UMSCC-10A, UMSCC-10B, UMSCC-22A, UMSCC-22B, UPCI-15B, 1483, 686LN, and FaDu) with expression levels in immortalised normal mucosal epithelial cells (Het1A) by RT–PCR. Expression of colXIa1 was detected in all nine HNSCC cell lines examined compared with the corresponding normal cell line, which did not express colXIa1 transcript (Figure 2). These results suggest that colXIa1 overexpression is, at least in part, due to increased expression in the transformed epithelial cell component of tumours.

Increased expression of colXIa1 in tumour-derived fibroblasts compared with fibroblasts from cancer-free subjects. It is well documented that, in response to wounds, normal fibroblasts secrete several types of collagen including colXIa1. Stromal...
expression of colXI1 is associated with malignancy in colorectal cancer (Fischer et al., 2001b). Higher levels of colXI1 were reported in fibroblasts associated with lung cancer compared with normal fibroblasts (Navab et al., 2011). HNSCC tumours are closely associated with fibroblasts. There are no reports on the levels of colXI1 expressed by stromal fibroblasts in the head and neck region. Primary HNSCC cancer-associated fibroblasts and fibroblasts from cancer-free subjects were grown out of tissue explants. We examined the levels of colXI1 in primary fibroblasts isolated from cancer-free patients and HNSCC explants. Our data demonstrate that the cancer-associated fibroblast express higher levels of colXI1 compared with fibroblasts from cancer-free patients (Figure 3).

**ColXI1 contributes to HNSCC proliferation, migration, and invasion.** Although colXI1 has been shown to correlate with tumour size in non-small cell lung cancer (Chong et al., 2006), the role of colXI1 in cell growth has not been examined in cancer cells. We hypothesised that, if colXI1 contributes to HNSCC proliferation, then knocking down colXI1 with siRNA would decrease cell proliferation *in vitro*. We transfected HNSCC cells with pooled siRNA targeting colXI1 or GFP as a control, collected cells on days 2, 3, 4, and 5, and performed RT–PCR. Gene inactivation was observed on day 5 (Figure 4A). Next, we tested individual siRNA and determined that siRNA B demonstrated efficient knockdown of colXI1 up to 48 h post transfection (Figure 4B).

The growth kinetics of cells transfected with siRNA- colXI1 duplex B was compared with cells transfected with non-targeting siRNA. colXI1 on transfection with siRNA duplex B significantly reduced cell proliferation compared with cells transfected with non-targeting control siRNA (*P* = 0.014) (Figure 5A). These results suggest that colXI1 contributes to cell proliferation in HNSCC cells *in vitro*.

ColXI1 expression has been reported to be 7.61-fold higher in the primary HNSCC tumour in the setting of metastasis to the
Type XI collagen, a minor fibril collagen, is a heterotrimer made up of a1(XI)-, a2(XI)-, and a3(XI) chains (Yoshioka et al., 1995) and accounts for ~3% of the adult cartilage matrix (Eyre et al., 2006). Molecules of collagen IX and XI stabilise collagen II, a major collagen, and limit its lateral growth (Blaschke et al., 2000). The role of Type XI collagen in the adult remains unclear, although recent evidence in numerous types of cancer implicates colXI1 in carcinogenesis (Vecchi et al., 2007; Badea et al., 2008; Ellsworth et al., 2009; Kim et al., 2010). Further, expression of colXI1 is reported to be accompanied expression of genes associated with epithelial-to-mesenchymal transition (EMT) (Anastassiou et al., 2011).

We previously reported that colXI1 was present in tumour tissue and was undetectable in corresponding normal adjacent tissue in a cDNA microarray analysis of tissue from nine HNSCC patients (Sok et al., 2003). Microarray of paired tumour and normal tissue from 36 patients with pancreatic ductal adenocarcinoma revealed greater than two-fold overexpression of colXI1 in tumour tissue compared with normal tissue (Badea et al., 2008). RT–PCR of paired tumour and normal tissue from 70 pairs of patients with non-small cell carcinoma demonstrated 3.4-fold higher expression of colXI1 in tumour tissue compared with normal tissue (Chong et al., 2006).

Similarly, RT–PCR of paired malignant and premalignant tissue from 21 patients with gastric cancer showed increased levels of colXI1 in malignant tissue (Zhao et al., 2009), and RT–PCR of polyps and normal tissue from 1 patient with familial adenomatous polyposis showed increased colXI1 expression in the polyps (Fischer et al., 2001a). Most of the reports on colXI1 in cancer represent correlative studies; thus, to our knowledge, this is the first investigation of the functional significance of colXI1 overexpression in any type of cancer.

Collagens in general are important components of the extracellular matrix. It has been shown that collagen cross linking increases ECM stiffness and promotes tumorigenesis in breast cancer and that reduction in collagen cross linking leads to a decrease in tumour incidence (Blaschke et al., 1994). We undertook the present study to determine the role of colXI1 in proliferation, migration, and invasion of head and neck cancer. Our results suggest that colXI1 expression is increased in HNSCC tumours and cell lines compared with levels in normal tissue or immortalised epithelial cells. Further investigation demonstrated that colXI1 contributes to the proliferation, migration, and invasion of head and neck cancer cells in vitro.

HNSCC carcinogenesis is characterised by a high incidence of synchronous and metachronous tumours, suggesting that molecular changes that are found in both the tumour and adjacent normal mucosa represent relatively early genetic changes (Bedi et al., 1996; Scholes et al., 1998). In this study, we found that colXI1 expression by RT–PCR was higher in tumour compared with normal tissue in paired samples in 16 of 23 lymph nodes compared with levels in a primarily HNSCC tumour that remains confined to the head and neck mucosa (Schmalbach et al., 2004). The role of colXI1 in cell migration or invasion has not been studied. To determine if colXI1 contributes to HNSCC migration in vitro, representative HNSCC cell lines (UMSCC-1 and FaDu) were transfected with colXI1 duplex B siRNA or the non-targeting control siRNA (siRNA-NTC). We also assessed invasion using a Boyden Matrigel chamber assay. ColXI1 knockdown in UMSCC-1 and FaDu cells resulted in reduced migration (P = 0.05) and invasion (P = 0.05), respectively (Figure 5B and C). Thus, colXI1 is an important facilitator of HNSCC progression.

Figure 3. Increased expression of colXI1 in tumour-derived fibroblasts compared with normal fibroblasts. Primary cancer-associated fibroblasts (TAF) isolated from HNSCC and fibroblasts isolated from oral mucosa of cancer-free patients (NNF) were assessed for colXI1 mRNA levels by RT–PCR. Expression of colXI1 was higher in the TAF lines compared with the NNF lines.

Figure 4. ColXI1 siRNA effectively reduces mRNA levels of colXI1. (A) HNSCC cells were transfected with pooled siRNA-targeted against the colXI1 gene or with control siRNA, which does not share sequences with the colXI1 gene. Cells were collected on days 2, 3, 4, and 5 and subjected to RT–PCR. Maximum gene inactivation was observed on day 5. RT–PCR amplification of the β-actin gene (lower panel) was used in each sample to monitor RNA levels and nonspecific RNA template degradation. (B) Cells were transfected with colXI1 duplex B siRNA. Efficient knockdown of colXI1 was achieved up to 3 days post transfection.
HNSCC patients, indicating that increased colXI1 expression is a later event in HNSCC carcinogenesis. Our findings are consistent with previous findings of increased colXI1 in tumour compared with normal tissue in paired samples from 70 patients with non-small cell lung carcinoma and in malignant tissue compared with pre-malignant tissue in paired specimens from 21 patients with gastric cancer (Zhao et al., 2009). Our attempts at generating a polyclonal antibody to colXI1 were not successful primarily because of the paucity of unique antigenic epitopes. Lack of specific antibodies limited our ability to assess protein levels of colXI1 in tissue and cell lines.

Because changes in the stromal tumour microenvironment are thought to contribute to invasion and spread of tumours (De Wever and Mareel, 2003), we compared the expression levels of colXI1 in cancer-associated fibroblasts and normal oral fibroblasts derived from cancer-free patients. We found that cancer-associated fibroblasts had higher levels of colXI1 compared with normal oral fibroblasts. The cancer-associated fibroblasts and normal oral fibroblasts used in these studies were primary cells derived from fresh tissue. These cells are cultured for up to 10 passages and retain their fibroblast morphology. However, it cannot be ruled out that HNSCC cells that have undergone epithelial-to-mesenchymal transition are present among the cancer-associated fibroblast cultures. The data suggest that changes in mesenchymal cells in the HNSCC microenvironment are associated with increased levels of colXI1, a finding that is at odds with Halsted et al. (2008), who found that colXI1 was downregulated in stroma surrounding breast cancer. In another study of breast cancer, however, higher levels of colXI1 expression in primary breast tumours than in corresponding metastatic lymph node tumours were reported and concluded that the overexpression of genes in the primary tumours were involved with extracellular matrix degradation and contributed to metastatic spread of the tumour (Ellsworth et al., 2009). Further, it has been proposed that interaction between breast and colon cancer cells with the microenvironment triggers expression of mesenchymal signature genes including colXI1 (Cheng et al., 2013).

We sought to determine if increased colXI1 expression was derived from the transformed epithelial cells and/or stromal cells in the tumour microenvironment. To date, there is no literature on colXI1 expression in cancer cell lines compared with normal cells. We compared colXI1 mRNA levels in nine validated, representative HNSCC cell lines using RT–PCR and found that expression was increased in all nine HNSCC cell lines, whereas the corresponding normal cells did not express the colXI1 transcript. Thus we conclude that both the tumour and stromal fibroblast cells contribute to the high levels of colXI1 in HNSCC tissue.

Although studies of gastric, lung, ovarian, and colorectal carcinomas have implicated the role of colXI1 overexpression in more advanced disease (Schmalbach et al., 2004; Vecchi et al., 2007; Zhao et al., 2009; Kim et al., 2010), only one study has correlated colXI1 and tumour size (Chong et al., 2006). Further, no direct investigation has been undertaken to examine the role of colXI1 in cellular proliferation of cancerous or normal cells. We postulated that by knocking down colXI1 in HNSCC cells in vitro, proliferation would decrease. We transfected a representative HNSCC cell line (UMSCC-1) with siRNA directed against colXI1 and demonstrated decreased colXI1 expression by RT–PCR. We then demonstrated that cellular proliferation of UMSCC-1 cells decreased in the siRNA-colXI1-transfected cells in comparison with the control. In contrast, normal Het1A cells did not display a decrease in proliferation after transfection with siRNA-colXI1. Therefore, these results suggest that colXI1 overexpression does, in fact, contribute to cellular proliferation in cancer cells and that knockdown of colXI1 abrogates cell growth in cancer cells but not normal cells.

Figure 5. ColXI1 contributes to HNSCC proliferation, migration, and invasion. (A) HNSCC cells UMSCC-1 were transfected with non-targeting control siRNA (NTC) or colXI1 siRNA. Cells were plated on day 2 post transfection, in the xCelligence DP E-plate and assessed for cell growth in real-time over 40 h. The cells were treated in quadruplicates. The line graph depicts cumulative data from four separate experiments. Error bars represent ± s.e.m. UMSCC-1 and FaDu cells transfected with siRNA-colXI1 duplex B or non-targeting control siRNA (NTC) was determined using a Boyden chamber (B) migration and (C) invasion assay. Migrated cells were counted 24 h post plating in four separate fields. Cumulative results are shown for wells treated in triplicate from three independent experiments (P = 0.05). Error bars represent ± s.e.m. (C) The invasion of UMSCC-1 and FaDu cells transfected with siRNA-colXI1 duplex B compared with cells transfected with non-targeting control siRNA (siRNA-NTC) was determined using a Boyden chamber Matrigel assay. Cumulative results from three independent experiments demonstrate a significant reduction in HNSCC invasion up on colXI1 siRNA treatment compared with the NTC siRNA control (P = 0.05).
Collagen type XI has been implicated in metastasis, with one study demonstrating higher levels of colXI in primary tumours of the head and neck that have metastasised to the lymph node compared with tumours that remain confined to the primary site (Schmalbach et al, 2004). Cellular migration and invasion are thought to contribute to metastasis. No studies to date have examined the role of colXI on cell motility of any type. We assessed the role of colXI in invasion and migration in UMSCC-1 and Het1A cells that had been transfected with siRNA-colXI. We demonstrated that knocking down colXI decreases migration and invasion in the HNSCC cell lines UMSCC-1 but not in the normal cell line Het1A. Thus, colXI not only contributes to proliferation but also to invasion and migration as well in cancer cells, with no effect on normal cells.

Patients with HNSCC, like many epithelial cancers, succumb to disease that has metastasised. The precise mechanisms of HNSCC metastasis, including invasion and migration remain incompletely understood. The results of the present study suggest that colXI has a role in mediating the proliferation, invasion, and migration of cancer cells, underscoring the need for further investigation of this collagen in carcinogenesis.

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