A potential link between desmoglein 3 and epidermal growth factor receptor in oral squamous cell carcinoma and its effect on cetuximab treatment efficacy

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Abstract

Desmoglein (DSG) 3 is overexpressed in oral squamous cell carcinoma (OSCC). Epidermal growth factor receptor (EGFR) inhibitor cetuximab is widely used for OSCC treatment. Several evidences suggest a correlation between DSG3 and EGFR in epidermal keratinocytes. EGFR inhibition has been shown to enhance cell-cell adhesion and induce terminal differentiation in epidermal cells. Thus, here we investigated the DSG3-EGFR interaction in OSCC and its effect on cetuximab treatment. Cell lines established from the primary tumor and metastatic lymph nodes of 4 OSCC patients and 3 commercial OSCC cell lines were used for the experiments. Cells from metastatic lymph nodes of each patient expressed increased DSG3 and EGFR than cells from the primary tumor in the same patient. Cetuximab treatment increased DSG3 expression by up to 3.5-fold in 7 of the 11 cell lines. A high calcium concentration increased the expression of DSG3 and EGFR in a dose dependent manner. Strikingly, a high-calcium-associated DSG3 induction enhanced cetuximab efficacy by up to 23% increase in cetuximab-low-sensitive cell lines. Our findings also suggest a correlation between DSG3 and EGFR in OSCC, and this affects cetuximab treatment efficacy.

Keywords

Desmoglein 3; Epidermal growth factor receptor; Oral squamous cell carcinoma; Cetuximab; Calcium

1. BACKGROUND

Desmoglein (DSG) 3, Ca^{2+}-dependent adehesion molecule, is an auto-antigen of the autoimmune blistering disease pemphigus vulgaris (PV) [1-3]. Several previous studies have confirmed upregulation of DSG3 in oral squamous cell carcinoma (OSCC) [4]. Epidermal growth factor receptor (EGFR) is also overexpressed in OSCC [5,6].

Recently, several lines of evidence suggest a correlation between DSG3 and EGFR in PV pathogenesis [7-9]. EGFR inhibitors blocked PV IgG-induced phosphorylation of EGFR [7]. Inhibition of EGFR blocked PV IgG-triggered DSG3 dysfunction [8]. AK23, a mouse monoclonal pathogenic anti-DSG3 antibody, induces EGFR activation and epidermal hyper proliferation in PV mouse model [10]. These findings suggest a cross-talk between DSG3 and EGFR in normal epidermal keratinocytes. However, there are few studies for this interaction in transformed keratinocytes.

Cetuximab (C-mab), anti-EGFR antibody, is widely used for OSCC treatment [11-13].

Previously, EGFR inhibition has been shown to stabilize the desmosome assembly and cell-cell adhesion, and induce terminal differentiation in epidermal cells [8,9,14]. On the other hand, high extracellular calcium (Ca) concentration is also known to induce the desmosome assembly and terminal differentiation [15,16], concomitant with an increase of DSG3 [17]. These findings and previous studies of DSG3-EGFR interaction led to the hypothesis that the combination of C-mab and high Ca switch may synergistically enhance therapeutic efficacy in OSCC.

2. QUESTIONS ADDRESSED

In this study, the potential link between DSG3 and EGFR expressions in 8 OSCC cell lines derived from the primary tumor and metastatic lymph nodes was first investigated [18]. Next, whether a high Ca concentration affects DSG3 expression and enhances efficacy of C-mab in OSCC was investigated.

3. EXPERIMENTAL DESIGN

See supplementary materials and methods.

4. RESULTS

4.1 Analysis of the potential link between DSG3 and EGFR expression in OSCC cell lines

Initially, expression of DSG3 and EGFR mRNA in cell lines from the primary tumor and metastatic lymph nodes of 4 OSCC patients was compared. Cells from metastatic lymph nodes showed significantly higher DSG3 expression compared to those from the primary tumor of the same patient (Figure 1A). EGFR expression in metastatic lymph node was also higher than that of the primary tumor (Figure 1B). Notably, one of the cells from the primary tumor (7P) did not express either DSG3 or EGFR at all (Figure 1A, B). When we statistically analyzed the direct correlation between DSG3 and EGFR expression in each cell line, we could not observe significant correlation (data not shown). Secondly, the effect of EGFR inhibition by C-mab on DSG3 expression in OSCC cell lines was evaluated. C-mab treatment upregulated DSG3 expression in 4 of the 8 cell lines (7LY, 17P, 58LY, and 60P) and in all commercial cell lines (HSC3,

HSC4, SAS) (Figure 1C). Since high extracellular Ca is known to increase DSG3 expression in normal keratinocytes [17], we investigated whether a high Ca concentration could upregulate DSG3 expression and affect EGFR expression. Although normal keratinocytes are maintained in low-Ca-medium (<0.3mM) and differentiated in high-Ca-medium (>1.0mM), SCC cell lines (including our OSCC cell lines) can be routinely propagated in media containing 1mM Ca [17]. Thus, we analyzed the effect of increased Ca concentration at 8mM and 16mM. Interestingly, both DSG3 and EGFR expression in cell line 7LY increased in a dose dependent manner (Figure 2A, B).

4.2 Effect of high Ca concentration on C-mab efficacy in OSCC cell lines

Previous reports demonstrated that EGFR inhibition enhanced cell-cell adhesion and induced terminal differentiation [8,9,14], and that high calcium switch led to DSG3 upregulation and terminal differentiation in epidermal keratinocytes [16,19]. These findings, combined with the results in our study that both C-mab treatment and high Ca concentration increased DSG3 expression in OSCC, led to the hypothesis that high Ca concentration enhances differentiation induction and the C-mab efficacy in OSCC. To validate this hypothesis, we investigated the effect of a high Ca concentration on C-mab

efficacy. When C-mab efficacy was evaluated by WST-1 cell proliferation assay, only 2 cell lines (17LY, SAS) were high-sensitive to C-mab treatment, whereas other cell lines were low-sensitive (Figure S1). Thus, 2 C-mab low-sensitive cell lines (7LY, HSC3) and 2 C-mab high-sensitive cell lines (17LY, SAS) were utilized for the following experiments. After 48 h culture at high Ca (8mM) concentration, DSG3 and EGFR expressions increased in C-mab low-sensitive cell lines, whereas not in C-mab high-sensitive cell lines (Figure 2C, D). A high Ca concentration enhanced C-mab efficacy only in C-mab low-sensitive cell lines with 43% reduction of viability (Figure 2E). The microscopic observation and the increment percentages of efficacy also showed an enhancement only in the C-mab low-sensitive cell lines, but not in C-mab high-sensitive cell lines (Figure 2F, G). These results indicate that high Ca switch increased the expressions of DSG3 and EGFR, and enhanced C-mab efficacy only in the C-mab low-sensitive cell lines.

5. CONCLUSIONS

In this study, our findings suggest, not a direct correlation, but a potential link between DSG3 and EGFR expressions in OSCC. Previous studies have reported that EGFR is implicated in the modulation of other isoforms of DSG (DSG1 and DSG2) in epidermal keratinocytes [20,21]. EGFR regulated endocytosis of DSG2 in squamous cell carcinoma, and promote its depletion from the cell membrane [22]. EGFR inhibition increased membranous DSG2 expression and cell adhesion [23,24]. These studies suggest that EGFR inhibition stabilizes desmosome assembly and cell-cell adhesion, whereas EGFR activation promotes desmosome disassembly and reduces cell-cell adhesion.

In our study, both C-mab treatment and a high calcium concentration upregulated DSG3 expression in OSCC cell lines. Previously, EGFR inhibition has been implicated in augmented cell adhesion and cell cycle arrest [14,23,25]. Jochen H, et al. demonstrated that EGFR inhibitors prevent proteolysis of desmosomal cadherin, resulting in an increase in DSG2 proteins in squamous cell carcinoma (SCC) [23]. Peus D, et al. showed that inactivation of EGFR by blocking antibodies induces expression of differentiation markers, keratin 1 and keratin 10, in epidermal keratinocytes [14]. Huang S M, et al. demonstrated that C-mab-treated SCC cells remained in the G0/G1 phase of the cell

cycle [25]. On the other hand, high calcium switch is known to induce DSG3 expression and terminal differentiation in epidermal cells [15,16]. Taking these previous reports and our findings into account, the therapeutic effect of C-mab was hypothesized to be due, in part, to differentiation induction and enhancement of cell-cell contact, and high-calcium-induced DSG3 expression is expected to enhance C-mab efficacy in OSCC. Findings from our study, specifically high Ca concentration increased DSG3 expression and enhanced C-mab efficacy in C-mab low-sensitive cell lines, also supported this hypothesis.

Since high Ca concentration also increased EGFR expression, the possibility of tumor progress is concerned. Recently, a direct DSG2-EGFR interaction on the cell surface, regulating the switch between adhesive and proliferative states in intestinal epithelial cells, was reported [26]. This study has identified a new role for DSG2 in regulating EGFR activity via a novel signaling complex consisting of DSG2 and EGFR. In DSG2 deficient cells, unbound EGFR was activated by ligand bindings that induced cell proliferation, whereas re-expression of DSG2 suppressed cell proliferation in DSG2 deficient cells. This direct DSG2-EGFR interaction was blocked by specific antibodies

against DSG2. Previously, the same DSG2-specific antibody has been shown to inhibit cell adhesion and increase intestinal epithelial cell proliferation [27]. Similarly, anti-DSG3 antibody induced EGFR activation and epidermal hyper proliferation [10]. Thus, DSG3 may also directly interact with EGFR at the cell borders to inhibit EGFR translocation to the nucleus and signaling towards cell proliferation. In line with this notion, we observed co-localization of DSG3 and EGFR at the cell surface in OSCC cell lines in high Ca concentration (Figure S2). Therefore, we speculated that high Ca-dependent DSG3 induction could inhibited EGFR signaling and overwhelmed the effect of increased EGFR expression in high Ca concentration.

In conclusion, this study provides a new insight into DSG3-EGFR interaction in OSCC, which is correlated with C-mab efficacy. From these findings, a novel therapeutic strategy, combination of C-mab and high calcium concentration, is proposed to overcome C-mab resistance in oral cancer treatment.

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Author contributions

M.M. performed the research, with exception of the experiment cited below. He was helped by Y.A. and supervised by S.T., T.N., and M.K.. M.K. designed the experiments. K.H. provided experimental support as well as lab space to perform the experiments. T.T. established OSCC cell lines derived from primary tumor and metastatic lymph nodes. M.M. performed the statistical analyses. M.M. provided the first draft of the manuscript, written by M.K. together with all co-authors.

Conflict of interest

The authors have declared no conflicting interests.

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Figure legends

Figure 1. Analysis of the potential link between DSG3 and EGFR expression in OSCC cell lines.

(A) Expression of DSG3 in 8 OSCC cell lines established from the primary tumor and metastatic lymph nodes of 4 OSCC patients (left panel). Box plot comparison of average DSG3 expressions of the primary tumor and metastatic lymph nodes (right panel). (B) EGFR expression in 8 OSCC cell lines established from the primary tumor and metastatic lymph nodes (left panel). Box plot comparison of average expressions (right panel). (C) DSG3 expression at 48 h after C-mab (5μg/ml) treatment in 8 patient's cell lines and 3 commercial cell lines. Results are presented as mean ± S.D. from three independent experiments. *, $P<0.05$. **, $P<0.01$.

Figure 2. Effect of high calcium concentration on C-mab efficacy in OSCC cell lines.

(A) DSG3 and (B) EGFR expressions in cell line 7LY after 48 h culture at different calcium concentrations (1, 8, 16mM). (C) DSG3 and (D) EGFR expressions in 2 C-mab low-sensitive cell lines (7LY, HSC3) and 2 C-mab high-sensitive cell lines (17LY, SAS) after 48 h culture at different calcium concentrations (1, 8mM). (E) Cell viability after 48 h C-mab (5μg/ml) treatment in 2 C-mab low-sensitive cell lines and 2 C-mab high-sensitive cell lines at different calcium concentration (1, 8mM). Values are normalized to cell viability of no treatment control. (F) Observation by light microscope after 48 h C-mab treatment in 2 C-mab low-sensitive cell lines and 2 C-mab high-sensitive cell lines at different calcium concentrations (1, 8mM). Cells were stained with crystal violet. Scale bars, 1mm. (G) Increment percentages of C-mab efficacy after high Ca switch (8mM) in 2 C-mab low-sensitive cell lines and 2 C-mab high-sensitive cell lines. Results are presented as mean ± S.D. from three independent experiments. *, $P<0.05$. **, $P<0.01$.

Supplementary figure legends

Figure S1. C-mab efficacy in OSCC cell lines.

Cell viability after 48 h C-mab (5μg/ml) treatment in 8 patient's cell lines and 3 commercial cell lines. Values are normalized to cell viability of no treatment control. Results are presented as mean ± S.D. from three independent experiments. *, $P<0.05$. **, $P<0.01$.

Figure S2. Colocalization of DSG3 and EGFR at the cell border in OSCC cell line.

Immunostaining for DSG3 (red) and EGFR (green) in cell line HSC3 at a high Ca concentration (8mM). DAPI (blue) was used to counterstain the nuclei. Scale bars, 10μm.

Figure 1

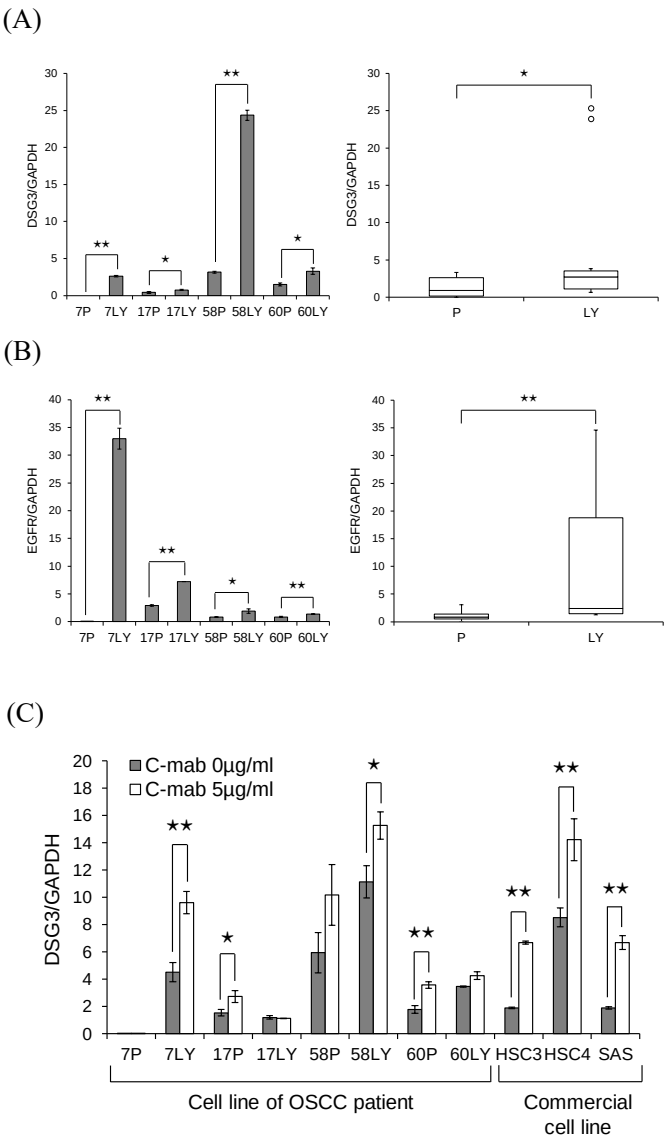
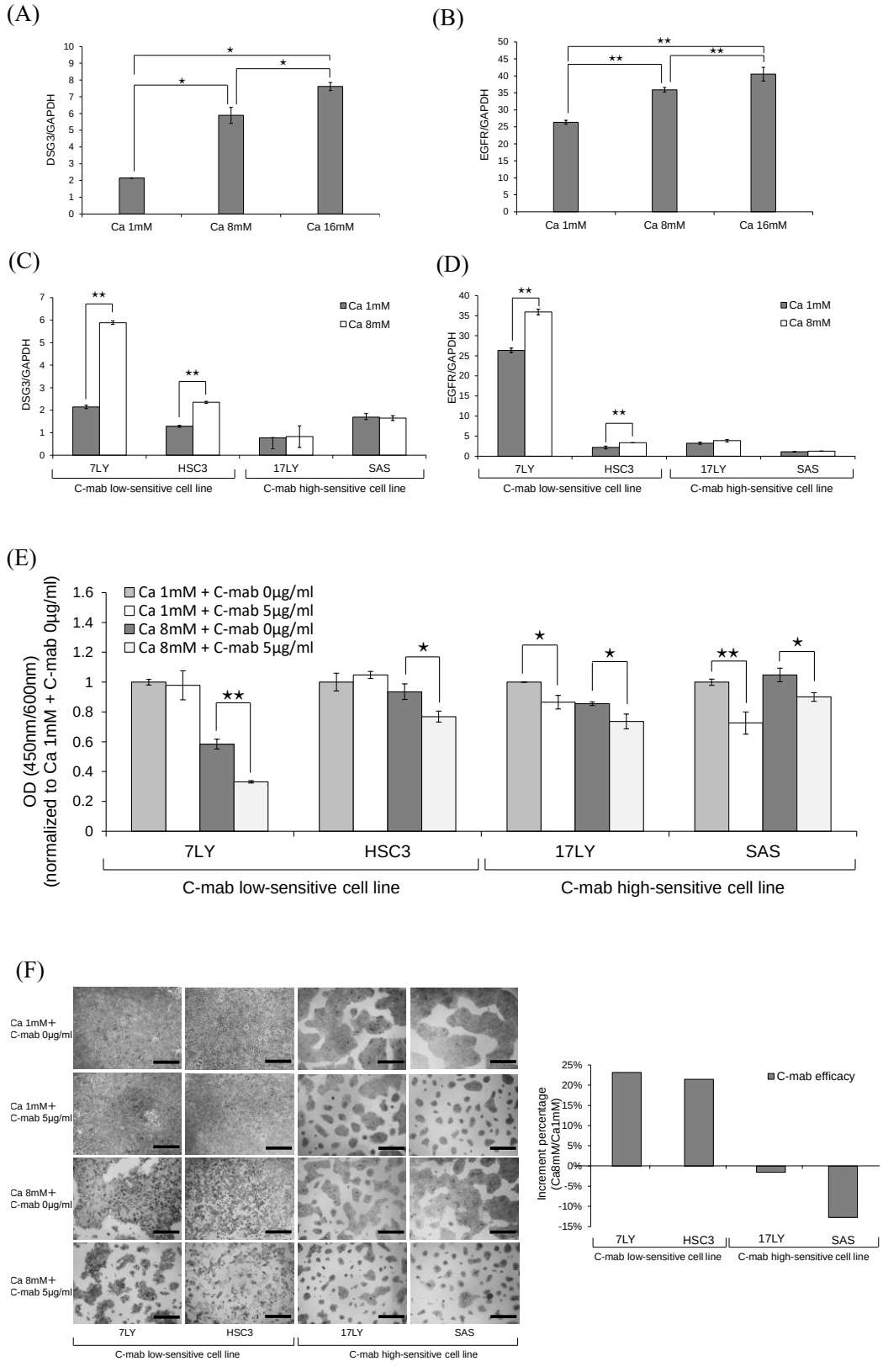

(A)
DSG3/GAPDH
7P 7LY 17P 17LY 58P 58LY 60P 60LY
P LY
(B)
EGFR/GAPDH
7P 7LY 17P 17LY 58P 58LY 60P 60LY
P LY
(C)
C-mab 0μg/ml
C-mab 5μg/ml
DSG3/GAPDH
7P 7LY 17P 17LY 58P 58LY 60P 60LY HSC3 HSC4 SAS
Cell line of OSCC patient
Commercial cell line

Figure 2


(A)
DSG3/GAPDH
Ca 1mM
Ca 8mM
Ca 16mM
(B)
EGFR/GAPDH
Ca 1mM
Ca 8mM
Ca 16mM
(C)
DSG3/GAPDH
Ca 1mM
Ca 8mM
7LY
HSC3
17LY
SAS
C-mab low-sensitive cell line
C-mab high-sensitive cell line
(D)
EGFR/GAPDH
Ca 1mM
Ca 8mM
7LY
HSC3
17LY
SAS
C-mab low-sensitive cell line
C-mab high-sensitive cell line
(E)
OD (450nm/600nm)
(normalized to Ca 1mM + C-mab 0μg/ml)
Ca 1mM + C-mab 0μg/ml
Ca 1mM + C-mab 5μg/ml
Ca 8mM + C-mab 0μg/ml
Ca 8mM + C-mab 5μg/ml
7LY
HSC3
17LY
SAS
C-mab low-sensitive cell line
C-mab high-sensitive cell line
(F)
Ca 1mM+
C-mab 0μg/ml
Ca 1mM+
C-mab 5μg/ml
Ca 8mM+
C-mab 0μg/ml
Ca 8mM+
C-mab 5μg/ml
7LY
HSC3
17LY
SAS
C-mab low-sensitive cell line
C-mab high-sensitive cell line
Increment percentage
(Ca8mM/Ca1mM)
C-mab efficacy
7LY
HSC3
17LY
SAS
C-mab low-sensitive cell line
C-mab high-sensitive cell line