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Loss of EGFR expression in oral squamous cell carcinoma is associated with invasiveness and epithelial-mesenchymal transition

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Background: In recent years, inhibition of EGFR signaling has emerged as new treatment strategy for oral squamous cell carcinoma (OSCC). The EGFR-directed inhibitor cetuximab is the only approved targeted therapy for the treatment of OSCC. The EGFR status may influence the response to cetuximab treatments.
Material and Methods: The subjects were 24 patients with primary OSCC who underwent surgical resection at the Department of Oral and Maxillofacial Surgery at Kanazawa University Hospital between 1998 and 2008. And, three human oral squamous cell carcinoma cell lines established from tumor biopsies with different grade of invasive abilities were used: OSC-20 (low grade invasive cells), OSC-19 (low grade invasive cells) and HOC313 (high grade invasive cells).
Results: In this study, by analyzing the immunomarker for EGFR, we found that it indicated positivity in 58.3% of all cases we analyzed, and the invasiveness was inversely correlated with the expression of EGFR. We quantified the expression level of EGFR and analyzed the correlation between EGFR expression and cetuximab sensitivity using three different grade invasive human OSCC lines. The EGFR expression in high grade invasive cells was significantly down-regulated compared to low grade invasive cells. There was no significant anti-proliferative effect in high grade invasive cells treated with various concentrations of cetuximab. The EMT-associated genes N-cadherin, vimentin and Snail were up-regulated in high grade invasive cells. Low grade invasive cells displayed characteristics of typical epithelial cells, including expression of E-cadherin and absence of N-cadherin, vimentin and Snail. TGF-beta induced this low grade invasive cell to undergo an EMT-associated gene switch that results in low EGFR expression.
Conclusions: These data suggested that loss of EGFR expression in OSCC was associated with EMT, and might have functional implications in tumor invasion and resistance to cetuximab treatments.
No conflict of interest.

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Anastomotic leakage in patients with rectal cancer after low anterior resection − our experience

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Background: The aim of this study was to evaluate the anastomotic leakage in patients after low anterior resection of the rectum.
Material and Methods: In the period 2000–2014, 369 patients with rectal cancer were operated in the General hospital “Sveti Vracevi” in Bijeljina. Anastomotic leakage was defined prior to the commencement of the study. Sphincter saving procedures (SSP) were performed in 82%, abdominoperineal resections (APR) in 14%, and resection of rectum with definitive stoma (Hartman procedure) in 4% patients.
Results: In the group of patients where SSP was performed (303 cases) there were 26% high colorectal anastomoses (8 cm from anal verge), 65% with low (4−8 cm from anal verge) and 9% with intrasphincteric coloanal anastomosis. Their mean age was 61 years, 32% of patients were ASA 3 or 4, and 17% of the operations were emergencies. Anastomotic leaks occurred in 8.5% (26/303) of anastomoses. The leak rate for intraperitoneal anastomoses was 7.8% (15/192) vs 10.8% for extraperitoneal anastomoses (11/101). Two-thirds of these leaks (17/26) were managed with re-operation or percutaneous drainage procedures. The minimum follow-up time is 24 months. Ten-year survival was 49%. Our analysis identified a covering stoma as independent predictor of a leak.
Conclusions: Low anterior resection combined with coloanal anastomosis provides good treatment for rectal cancers. The creation of a diverting stoma proximal to a high risk anastomosis minimizes the severe consequences of a leak but does not reduce the incidence of leak itself.
No conflict of interest.

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Inhibition of epithelial mesenchymal transition in response toTGF-β1 silencing associated with metformin in canine mammary tumor cell line

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Background: Epithelial mesenchymal transition (EMT) is the process in which cancer cells from primary tumors pass through a phenotypic conversion to invade and migrate, generating metastases in distant tissues. EMT can be induced by growth factors such as transforming growth factor beta (TGF-β). Overexpression of TGF-β has been implicated in cell migration and invasion in many cancers, including breast cancer. Metformin, an important medicine for diabetics, has been considered an inhibitory agent of EMT for supplying the expression of transcription factors in breast cancer cells. The aim of this study was to inhibit the EMT process by TGF-β silencing associated with metformin treatment in canine mammary tumor cell line.
Material and Methods: Canine metastatic mammary tumor cell line (CF-41) was cultured and cell viability was measured after treatment with different concentrations of metformin by MTT assay. Small interfering RNA constructs targeting TGF-β1 (TGF-β1si) were validated and used to develop clonal derivatives of the CF-41 cell line. Subsequently it was performed a cell migration assay, associated with metformin 5 mM treatment for 42 hours. Furthermore, the proteins E-cadherin and N-cadherin were quantified by immunofluorescence after metformin treatment. In an in vivo study, unmodified or TGF-β1 siRNA-expressing CF-41 cells were injected in the right inguinal region of nude athymic female mice and treated with metformin (200 mg/kg) for 4 weeks. At the end, mice were sacrificed and the lungs were collected to determine histologically reducion of metastases occurrence. After the detection of metastases in the lungs, N-cadherin, E-cadherin, Vimentin, Claudin-7 and Twist markers were analyzed by immunohistochemistry.
Results: The migration and invasion rate was lower in CF-41 TGF-β1si cells when compared with parental CF-41 cells and this inhibition was even more significant when associated to metformin treatment ($p < 0.05$). Immunofluorescence analyzes demonstrated that metformin treatment reduced N-cadherin expression and increased E-cadherin expression in CF-41 and CF-41 TGF-β1si cells when compared with untreated cells. Corroborating with in vitro results, in vivo study found less lung metastases in animals that received CF-41 TGF-β1si cells. In addition, tumor induction with CF-41 TGF-β1si cells could decrease expression of mesenchymal markers N-cadherin, vimentin and twist, and increase the expression of epithelial markers E-cadherin and Claudin-7 in lung metastases. Also, it was observed further reduction of mesenchymal EMT markers when TGF-β1si cells were combined with metformin treatment.
Conclusion: This study confirms the benefits of TGF-β1 silencing added to metformin as potential therapeutic agents for breast cancer patients, by blocking EMT process and sequential metastatic potential.
No conflict of interest.

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Implication of IL-7 in mesothelial paracellular resistance, migration and interaction with colorectal cancer cells

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Background: Dissemination of tumour cells to surrounding tissues is a major factor in defining patient outcomes. In addition to systemic and vascular spread, cancer cells may also form transcoelomic metastasis to local tissues such as the mesothelial lining. This is often seen in cancers such as ovarian and colorectal but can also occur as a result of cell seeding during surgical procedures to remove the primary tumour. Novel strategies and methodologies to combat such transcoelomic metastasis are required. The current study explores the role of Interleukin-7 on mesothelial cells and their interaction with colorectal cancer cells.
Methods: Omentum tissue was obtained from patients following surgery in accordance with ethical approval. Primary mesothelial cells were generated from the omentum tissues under aseptic techniques. Electric cell substrate impedance sensing (ECIS) system, together with 96W1E arrays, was used