ORIGINAL ARTICLE

INVERSE CORRELATION BETWEEN SERUM PGE2 AND T CLASSIFICATION IN HEAD AND NECK CANCER

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Abstract: *Background.* Prostaglandin E2 (PGE2) serum levels have been shown previously to be increased in tumor bearing mice as well as in patients with solid tumors; however, the impact on the course or stage of disease has not been shown. We hypothesized that PGE2 is strictly required for aggressive and especially early-stage tumors of the head and neck to provoke invasion and angiogenesis.

Methods. We analyzed the serum PGE2 levels of 100 patients with head and neck squamous cell carcinoma of different stages before and 1 year after treatment and compared the results with the serum levels of 40 healthy donors and the secretion profile of 8 different squamous cell carcinoma cell lines.

Results. Our investigations showed a statistically significant inverse correlation between PGE2 serum levels and tumor stage. Furthermore, this effect has been reflected by the results of our cell culture analyses, which showed an inversely regulated PGE2 secretion into the medium during the process of proliferation. Interestingly, the serum levels of PGE2 were significantly downregulated 1 year after successful treatment.

Conclusions. We conclude that PGE2 serum level as an indicator for early-stage cancer of the head and neck may function as a tumor marker during the follow-up period.
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In recent years cyclooxygenase 2 (COX-2) inhibitors have been increasingly investigated for their potential to inhibit growth of solid tumors. A few studies provided evidence that COX-2 inhibition reduced tumor cell growth remarkably.[1–4] COX-2 is a key enzyme in the conversion of arachidonic acid to prostaglandins. The overexpression of COX-2 has been reported in head and neck squamous cell carcinomas.[5] Prostaglandin E2 (PGE2), the end product of COX-2-induced catalysis, autoamplifies the COX-2 expression in solid tumors.[6] It downregulates the apoptotic cascade,[7] activates the Ras signal pathway,[8] induces Ca^{2+} influx and cyclic guanosine monophosphate formation,[9] and increases the cyclic adenosine monophosphate (cAMP) content of cancer cells.[10] Production of PGE2 is augmented by epidermal growth factor (EGF).[11] The epidermal growth factor receptor (EGFR), conversely, is transactivated by PGE2.[12] PGE2 expression and COX-2 activity is associated with cancer cachexia, humoral hypercalcaemia of malignancy, and tumor angiogenesis.[13–17]

PGE2 serum levels have been shown to be increased in tumor-bearing mice[16] as well as in patients with solid tumors[15,18,19] without showing

impact on the course or stage of disease. Increased levels of PGE2 have been detected in both premalignant and malignant lesions, including squamous cell carcinoma of the oral cavity.[20] This increase results from the overexpression of COX-2, the inducible form of COX. Several lines of evidence, beyond the finding of elevated levels of PGE2 in tumors, suggest that COX enzymes contribute to the development of oral cancer.[21] COX can convert polycyclic aromatic hydrocarbons in tobacco smoke to reactive metabolites, which form mutagenic DNA adducts.[21] This in turn could lead to genomic instability, as expressed in the molecular risk marker aneuploidy, which may explain why COX-2 overexpression predicts a shorter survival in patients with head and neck cancer.[5] Furthermore, PGE2 tumor levels correlate with vascularization of the tumor and VEGF expression,[5] and it enhances cell motility and invasiveness.[22]

We therefore hypothesized that PGE2 is strictly required for aggressive and especially early-stage tumors of the head and neck to provoke invasion and angiogenesis. We analyzed the serum PGE2 levels of 100 patients with head and neck squamous cell carcinoma of different stages before and 1 year after treatment and compared the results with the serum levels of 40 healthy donors and the secretion profile of 8 different squamous cell carcinoma cell lines.

MATERIALS AND METHODS

Cell Cultures. Ten thousand cells of each of 7 human squamous cell carcinoma cell lines (A431, Detroit 562, SCC-1624; American Type Culture collection, ATCC/UM-SCC 24, UM-SCC 10b, UM-SCC 5, UM-SCC 33; Tom Carey, University of Michigan) and human fibroblasts (HFF-1; ATCC) were seeded into 10 mL cell culture flasks after adding 10 mL of Quantum Medium (PAA Laboratories GmbH, Cölbe, Germany). After 24, 72, 96, and 120 hours, the cell culture medium was changed and the removed medium was stored at $<-70^{\circ}$C. Medium from fibroblasts, fetal calf serum, and quantum medium alone served as controls.

Human Serum. Patients ($n = 100$) with diagnosed squamous cell carcinoma of the head and neck were asked to give their consent for collecting a blood sample before and 1 year after treatment for the tumor. Serum (10 mL) of each patient was collected at each time point. We chose 25 patients for each T classification according to the TNM classification of the Union Internationale Contre le Cancer (UICC).

Also 40 healthy donors with a diverse risk profile (18 heavy smokers, 22 nonsmokers) and of different ages (21–59 years) were asked to give their consent for this investigation. Serum (10 mL) was collected from each donor.

Prostaglandin E2 Measurement. Cell culture supernates and serum samples were centrifuged for 15 minutes at 1000*g* within 30 minutes of collection. The prostaglandin synthetase inhibitor indomethacin was added with a concentration of 10 μg/mL to the sample, and a 10-fold dilution was prepared by adding 450 μL assay buffer ED1 (R&D Systems, Minneapolis, MN) to 50 μL sample. For extraction of the sample acidification, 50 μL HCL was added to 1 mL sample. After 15 minutes, precipitates were removed using a microcentrifuge for 2 minutes. The sample was then applied to a C_{18} reverse phase column with a flow rate of 0.5 mL/minutes. After washing the column with 10 mL deionized ice water, followed by 10 mL 15% ethanol and 10 mL hexane, the sample was eluted from the column by the addition of 10 mL of ethyl acetate. Finally, prior to running the immunoassay, samples were evaporated under a stream of nitrogen and were added to 250 μL assay buffer ED1.

PGE2 concentration was then determined by using the PGE2 high sensitivity ELISA kit (R&D Systems). The intensity of the yellow dye from the PGE2 HS antibody solution was inversely proportional to the concentration of PGE2 in the sample. The ED1 assay buffer and the cell culture medium, respectively, served as standards. For each set of samples, a separate standard curve was generated.

Statistics. Statistics were performed using the SPSS™ Software v 9.0 (SPSS, Chicago, IL). The nonparametric Kruskal-Wallis, Wilcoxon, and Mann–Whitney *U* tests were performed for analyzing multiple independent samples, 2 independent samples, and 2 dependent samples, respectively. Results were assumed to be significant with a p value below .05.

RESULTS

Secretion of Prostaglandin E2 by Squamous Cell Carcinoma Cell Lines. Ten thousand cells of each cell line were seeded into medium filled flasks.

After 24 hours and on 3 further time points until the cells reached a complete adherence, medium was changed and analyzed for PGE2 content. Fibroblasts, pure medium, and fetal calf serum served as controls.

As shown in Figure 1, PGE2 secretion was highest at the beginning of proliferation and during the exponential growth phase. At the end point, when each cell had contact to another cell, PGE2 secretion was lowest. These results were significantly different when compared with the control ($p < .001$).

Serum Prostaglandin E2 Levels of Head and Neck Cancer Patients and Healthy Donors. Serum PGE2 levels at the time of diagnosis were significantly higher in the serum of patients with head and neck carcinomas than in the serum of healthy donors (Figure 2, $p < .001$). Interestingly, there was no statistically significant difference between PGE2 serum levels of healthy smokers and nonsmokers. One year after treatment, the serum levels of the patients did not differ significantly from the control (Figure 2, $p > .56$). As shown in Figure 3, the total amount of serum PGE2 decreased from T1 to T4 classification. At the time of diagnosis, serum PGE2 was significantly higher in patients with T1 tumors when compared with the PGE2 serum levels of patients with T2, T3, or T4 tumors (Figure 3, $p < .001$). Also serum levels of patients with T2 and T3 tumors were significantly higher compared with the PGE2 levels

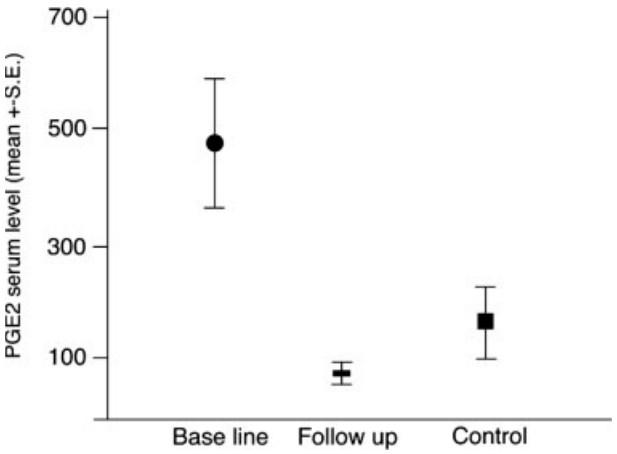

FIGURE 2. Serum levels of all patients at the time of diagnosis and 1 year after successful treatment when compared with the control group of healthy donors. Serum PGE2 levels at the time of diagnosis were significantly higher in the serum of patients with head and neck carcinomas than in the serum of healthy donors ($p < .001$). One year after treatment, the serum levels of the patients did not differ significantly from the control ($p = .56$). The graph shows the mean PGE2 level (pg/mL) in the serum ± SE.

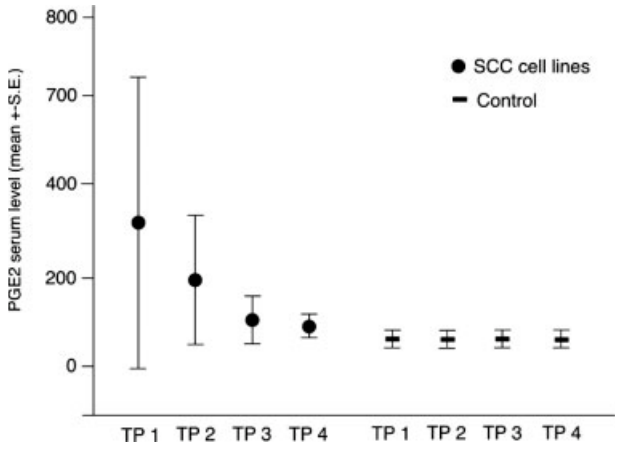

FIGURE 1. Secretion profile of different squamous cell carcinoma cell lines and fibroblasts. Cell culture medium and pure fetal calf serum served as controls. Ten thousand cells of each cell line were seeded into medium-filled flasks. After 24, 72, 96, and 120 hours, medium was changed and analyzed for PGE2 content (pg/mL). Fibroblasts, pure medium, and fetal calf serum served as controls. PGE2 secretion was highest at the beginning of proliferation and during the exponential growth phase. At the end point, when each cell had contact with another cell, PGE2 secretion was lowest ($p < .001$). The graph shows the mean PGE2 level (pg/mL) in the cell culture medium ± SE.

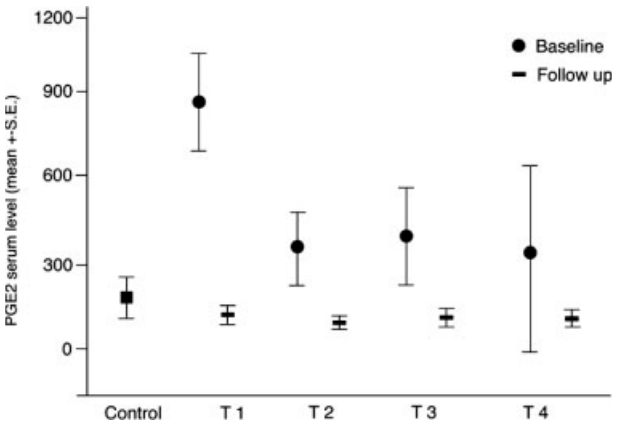

FIGURE 3. Serum levels of the patients divided into 4 groups according to their initial T classification at the time of diagnosis and 1 year after successful treatment when compared with the control group of healthy donors. The total amount of serum PGE2 decreased from T1 to T4. At the time of diagnosis, serum PGE2 was significantly higher in patients with T1 tumors when compared with the PGE2 serum levels of patients with T2, T3, or T4 tumors ($p < .001$). Serum levels of patients with T2 and T3 tumors were significantly higher compared with the PGE2 levels of T4 patients and the control group ($p < .001$). The PGE2 serum levels of patients with T4 tumors did not differ.

of T4 patients and the control group (Figure 3, $p < .001$). The PGE2 serum levels of patients with T4 tumors, however, did not differ significantly when compared with the control group (Figure 3, $p < .34$).

DISCUSSION

For the first time to our knowledge, PGE2 serum levels of patients with squamous cell carcinoma of the head and neck have been shown to correlate with the clinical stage of head and neck tumors. As described recently, COX-2 overexpression predicts a shorter survival in patients with head and neck cancer, and PGE2 tumor levels correlate with vascularization of the tumor and VEGF expression.[5] It furthermore enhances cell motility and invasiveness.[20] Therefore, it seems to be required for invasive proliferation of tumor cells.

Our investigations showed a statistically significant inverse correlation between PGE2 levels and tumor stage (see Figure 3). Furthermore, this effect has been reflected by the results of our cell culture analyses, which showed an inversely regulated PGE2 secretion into the medium during the process of proliferation (Figure 1). We therefore hypothesize that during the phase of early tumor growth, PGE2 secretion is necessary for interacting with surrounding tissue and provoking neovascularization, which is strictly recommended for further invasion.[5,20] This may explain, why advanced tumors (T3 and T4) do not secret high levels of PGE2, because in those cases major neovascularization has already taken place. Interestingly, the serum levels of PGE2 were significantly downregulated 1 year after successful treatment. We therefore suggest that the PGE2 serum levels are tumor dependent. The cross-related interactions between the EGFR pathway and COX-2-mediated pathways,[11] together with the fact that cAMP levels in cancer cells are increased by PGE2,[10] additionally indicates that head and neck carcinomas, which overexpress the EGFR, strictly require PGE2 during early proliferation. Our study supports new evidence for the need of COX-2 inhibition in cancer treatment and may give an option to choose the right patients to treat with.

We further conclude that PGE2 serum level as an indicator for early-stage cancer of the head and neck may function as a tumor marker during the follow-up period.

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