Journal of Cancer Research and Clinical Oncology (2019) 145:313–319
https://doi.org/10.1007/s00432-018-2785-3

ORIGINAL ARTICLE – CANCER RESEARCH

The role of EP3-receptor expression in cervical dysplasia

Anna Hester[1] · Manuel Ritzer[1] · Christina Kuhn[1] · Elisa Schmoeckel[2] · Doris Mayr[2] · Thomas Kolben[1] · Christian Dannecker[1] · Sven Mahner[1] · Udo Jeschke[1] · Theresa Maria Kolben[1]

Received: 9 October 2018 / Accepted: 27 October 2018 / Published online: 7 November 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Purpose Prostaglandin-mediated inflammatory reactions play a major role in different cancers. Prostaglandin E2-receptor 3 (EP3) expression correlates with FIGO stages in cervical cancer and has been shown to be an independent prognostic factor for overall survival. EP3 expression levels in cervical intraepithelial neoplasia (CIN) as the precursor lesion of cervical cancer are currently unknown.

Methods EP3 expression was analyzed by immunohistochemistry in 124 patient samples (CIN 1–3 and healthy controls) using the IR-scoring system. Expression levels were correlated with clinical outcome to assess for prognostic relevance in patients with CIN 2. Data analysis was performed using Kruskal–Wallis and Mann–Whitney *U* test.

Results EP3 expression levels significantly correlated with different grades of cervical dysplasia. Median EP3-IRS in healthy cervical tissue was 12 ($n=13$) compared to 9 in CIN 1 ($n=38$; $p=0.031$ vs. healthy control), 6 in CIN 2 ($n=45$; $p<0.001$ vs. CIN 1) and 4 in CIN 3 ($n=28$, $p=0.008$ vs. CIN 2). The percentage of EP3 expressing cells in CIN 2 lesions was significantly lower in progressive than in regressive cases (mean percentage of EP3 positive cells in progress: 3.8%, $n=18$; in regress: 9.3%, $n=20$; $p=0.040$).

Conclusion EP3 expression significantly decreases with higher grades of cervical intraepithelial neoplasia—which is in line with published IR scores in cervical cancer patients—and seems to be a prognostic marker for regression or progression of CIN 2 lesions. Our findings support the importance of the prostanoid pathway in cervical cancer and could help to identify targets for future therapies.

Keywords EP-receptor · EP3 · Prostaglandin E2 · CIN · Cervical intraepithelial neoplasia · Cervical cancer

✉ Udo Jeschke
udo.jeschke@med.uni-muenchen.de

Anna Hester
anna.hester@med.uni-muenchen.de

Manuel Ritzer
ritzer.manuel@gmx.de

Christina Kuhn
christina.kuhn@med.uni-muenchen.de

Elisa Schmoeckel
elisa.schmoeckel@med.uni-muenchen.de

Doris Mayr
doris.mayr@med.uni-muenchen.de

Thomas Kolben
thomas.kolben@med.uni-muenchen.de

Christian Dannecker
christian.dannecker@med.uni-muenchen.de

Sven Mahner
sven.mahner@med.uni-muenchen.de

Theresa Maria Kolben
theresa.kolben@med.uni-muenchen.de

[1] Department of Obstetrics and Gynecology, University Hospital, LMU Munich, Marchioninistr. 15, 81377 Munich, Germany

[2] Department of Pathology, LMU Munich, Thalkirchnerstrasse 36, 80337 Munich, Germany

Introduction

Cervical cancer represents the second most common malignancy in women worldwide. Per year about half a million women are diagnosed, with more than 250,000 women still die from this disease (Torre et al. 2015). Incidence of cervical cancer has dramatically dropped after introduction of Pap smear screening. The goal of this screening was the detection of cervical intraepithelial neoplasia (CIN) as a direct precursor lesion of cervical cancer. CIN lesions are categorized in three grades (CIN 1–3) according to the amount of epithelium involved in dysplastic changes. Main risk factor for the development of cervical intraepithelial neoplasia, as well as cervical cancer is the persistence of high-grade human papillomavirus (HPV) infection. CIN 1 lesions are considered to be transient effects of HPV infection and typically have a high likelihood of spontaneous regression (Brummer et al. 2006; Cox et al. 2003; Moscicki et al. 2004; Trimble et al. 2005). Therefore, a watchful waiting strategy is justified in most cases which is in accordance to international guidelines (Jordan et al. 2009; Massad et al. 2013). In contrast, CIN 3 lesions have a significant risk of progression to invasive cervical cancer and are, therefore, surgically managed by conisation. Management of patients with CIN 2 lesions is currently discussed controversially (Wilkinson et al. 2015). According to the widely used Bethesda classification and the Lower Anogenital Squamous Terminology Project, CIN 2 and CIN 3 lesions are both classified as high-grade dysplasia, and therefore, both require surgical management (Darragh et al. 2012). However, growing evidence supports the idea of a more CIN 1 like appearance of CIN 2 lesions, which could justify a conservative approach at least in certain patient populations, e.g. young patients of childbearing age (Kolben et al. 2017). Benefit of a non-surgical management would be the prevention of pregnancy-associated risks like preterm labour and cervical insufficiency, which is a typical complication occurring in patients after cervical conisation (Arbyn et al. 2008; Jin et al. 2014; Kyrgiou et al. 2006). However, there is no established prognostic biomarker that enables the prediction of progression or regression in patients with CIN 2 lesions.

The prostaglandin E2-receptor EP3 has been shown to be an independent negative prognostic factor in cervical cancer patients. Expression levels correlate with tumor stage as well as clinical outcome (Heidegger et al. 2017). We, therefore, hypothesized that EP3 receptor expression levels could be associated with different grades of cervical intraepithelial neoplasia lesions and moreover could help to predict regression or progression in patients with CIN 2 to avoid surgery with its associated complications in these patients.

Methods

Tissue samples

Cervical tissue ($n = 140$) of patients treated between 2002 and 2014 in the Department of Gynecology and Obstetrics, Ludwig-Maximilians-University of Munich, Germany was collected. Successful EP3-staining could be obtained in 124 cases; patients had either no dysplasia ($n = 13$), CIN 1 ($n = 38$), CIN 2 ($n = 45$) or CIN 3 ($n = 28$). Diagnosis as well as grade of dysplasia were confirmed by a second pathologist. Only patients who had a follow-up visit with either histologically confirmed progress ($n = 23$) or regress ($n = 22$) were selected for CIN 2 samples. Median follow-up interval was 5 months (range 1–27 months). All CIN patients were tested positive for high-risk HPV (Hybrid capture 2, Qiagen) on their first visit. Tissue analyzed within this study had initially been collected for routine histopathological diagnostics and all diagnostic procedures had been completed. Patient data were fully pseudonymized and analyses were performed according to the standards set in the declaration of Helsinki 1975 and its amendment of Seoul, 2008. Informed consent was obtained prior to study participation. Study design was approved by the Ethics Committee of the Ludwig-Maximilians-University (Munich, Germany).

Immunohistochemistry

Cervical tissue samples were paraffin-embedded and immunohistochemistry was performed as previously described (Heidegger et al. 2017; Semmlinger et al. 2018). Samples of metastatic ovarian cancer tissues were used for positive and negative (without antibody) control. Samples were stained with primary anti-EP3-antibodies (polyclonal rabbit IgG, Abcam, Cambridge, UK) and detected via polymer-method (ZytoChem Plus HRP Polymer System mouse/rabbit, Zytomed Systems Berlin, Germany) and the chromogen diaminobenzidine (Dako, Hamburg, Germany). A well-established immune-reactivity scoring system (IR-score, IRS or Remmele score) was employed to quantify immunostaining in a semi-quantitative manner using a Leitz (Wetzlar, Germany) microscope. The IRS multiplies the intensity of the staining (0 = no, 1 = weak, 2 = moderate, 3 = strong staining) with the percentage of positive cells (0 = no staining, $1 \leq 10\%$ positive cells, 2 = 11–50% positive cells, $3 \geq 50\%$ positive cells). In CIN 2 samples, the absolute percentage of EP3 expressing cells was determined separately. The researcher analyzing the percentage of EP3 expressing cells in CIN2 was blinded regarding progressive vs. regressive course of the disease.

Statistical analysis

Statistical analysis was performed using SPSS 25 (PASW Statistic, SPSS Inc., IBM, IL, USA). Comparative analysis of EP3 expression between healthy cervical tissue and the different grades of cervical dysplasia was performed using the nonparametric Kruskal–Wallis rank-sum test and Mann–Whitney *U* test. p values ≤ 0.05 were considered as statistically significant. Figures were designed with SPSS 25 and Microsoft Power Point 2016.

Results

EP3 is expressed in healthy cervical tissue and in all grades of cervical dysplasia

EP3 expression could be shown in healthy cervical tissue and in all grades of cervical dysplasia. In Fig. 1, examples of EP3 staining in cervical dysplasia (a) compared to EP3 staining in healthy cervical tissue (b), positive (c) and negative (d) controls are shown.

EP3 expression decreases with increasing grade of cervical dysplasia

To examine differences in EP3 expression, we compared EP3-IR-scores between the groups of cervical tissue samples as displayed in Fig. 2. EP3 expression decreased significantly with increasing grade of cervical dysplasia; this difference was statistically significant comparing each grade of dysplasia to the next higher one. While healthy cervical tissue showed a median IRS of 12, median EP3-IRS was 9 in CIN 1 (Mann–Whitney *U* test vs. healthy controls: $p = 0.031$), but only 6 in CIN 2 (Mann–Whitney *U* test vs. CIN1: $p < 0.001$) and CIN 3 showed a median EP3-IRS of only 4 (Mann–Whitney *U* test vs. CIN 2: $p = 0.008$). EP3-IR-scores are shown in Fig. 2a, exemplary stainings for all grades of CIN are shown in Fig. 2b–e.

Percentage of EP3 positive cells is lower in CIN 2 lesions which show progressive course of the disease

To evaluate if EP3 expression might be a prognostic factor of progressive or regressive course in cervical dysplasia, we compared EP3 expression between CIN 2 cases with histologically confirmed regress or progress. We could not show a statistically significant difference of IR-scores of

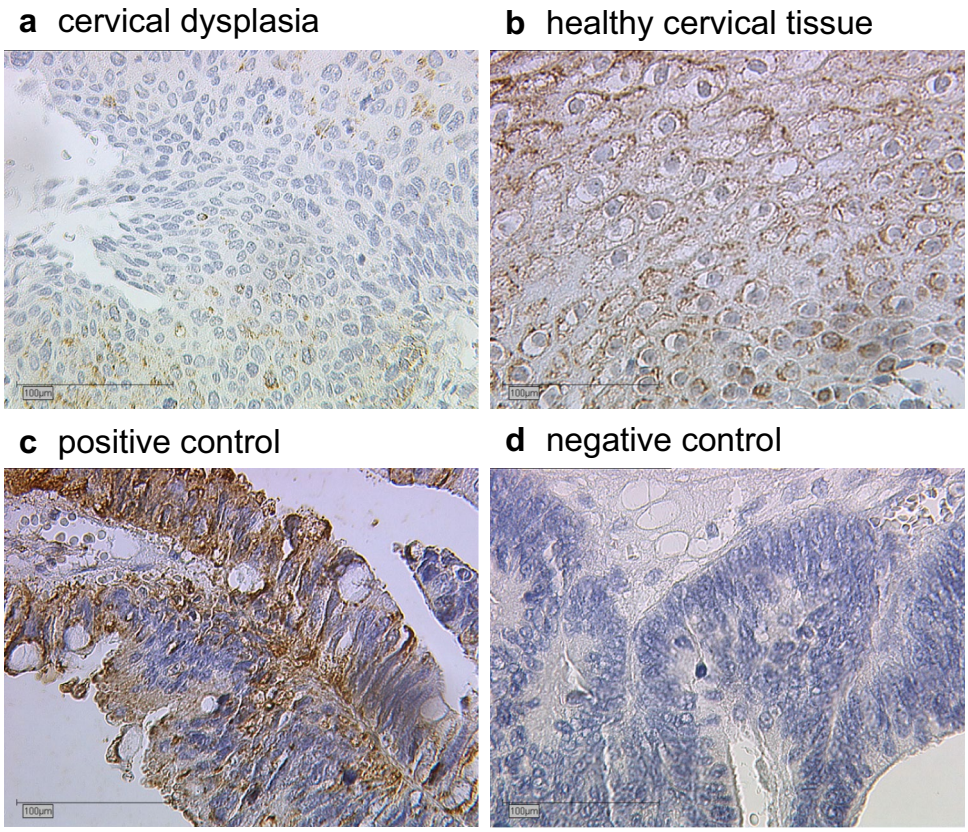
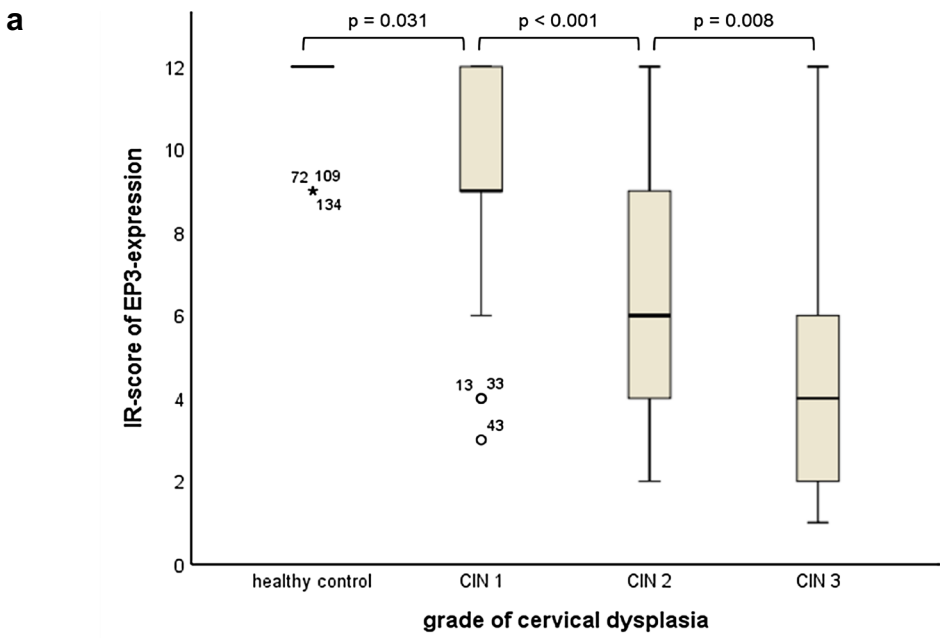


Fig. 1 EP3-staining in cervical tissue and controls. Exemplary images of immunohistochemical EP3 staining in cervical dysplasia (**a**) as well as EP3 staining in healthy cervical tissue (**b**), positive control of ovarian cancer metastasis in the colon (**c**) and negative control of serum without antibody on ovarian cancer metastasis in the colon (**d**) are shown



n	13	38	45	28
Median EP3-IRS	12	9	6	4

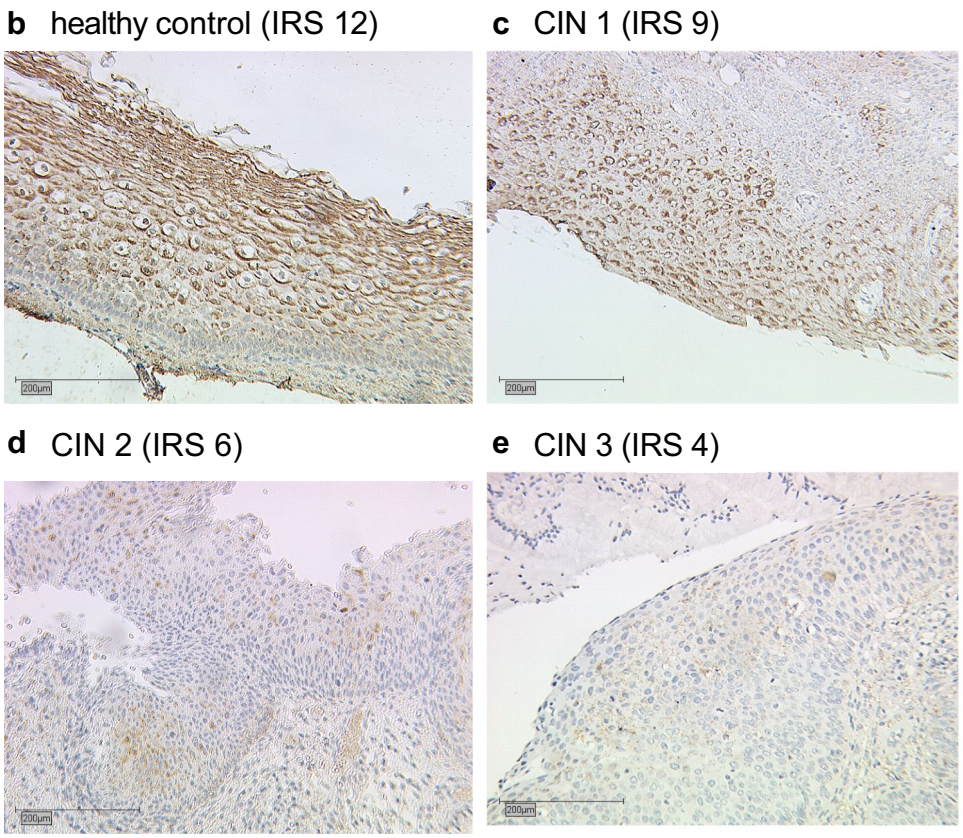


Fig. 2 EP3-expression in healthy cervical tissue and cervical dysplasia. Boxplots of EP3-IR-scores in healthy controls of cervical tissue, in CIN 1, CIN 2 and CIN 3 are shown (**a**). Number of samples analyzed in each group (*n*) and median values of EP3-IR-score are displayed in the table below the graph. Exemplary images of immunohistochemical EP3 staining in healthy controls (**b**), CIN 1 (**c**), CIN 2 (**d**) and CIN 3 (**e**) are shown below the table

EP3 expression between regressive and progressive CIN 2 cases (in both groups: median-IRS 6. Mann–Whitney *U* test, $p = 0.195$, data not shown). However, the mean percentage of cells stained positive for EP3 differed significantly in both groups: While 9.3% of cells of CIN 2 samples with regressive disease showed positive EP3 staining, only 3.8% of cells

were positive for EP3 in progressive CIN 2 cases (Fig. 3, Mann–Whitney *U* test, $p = 0.04$).

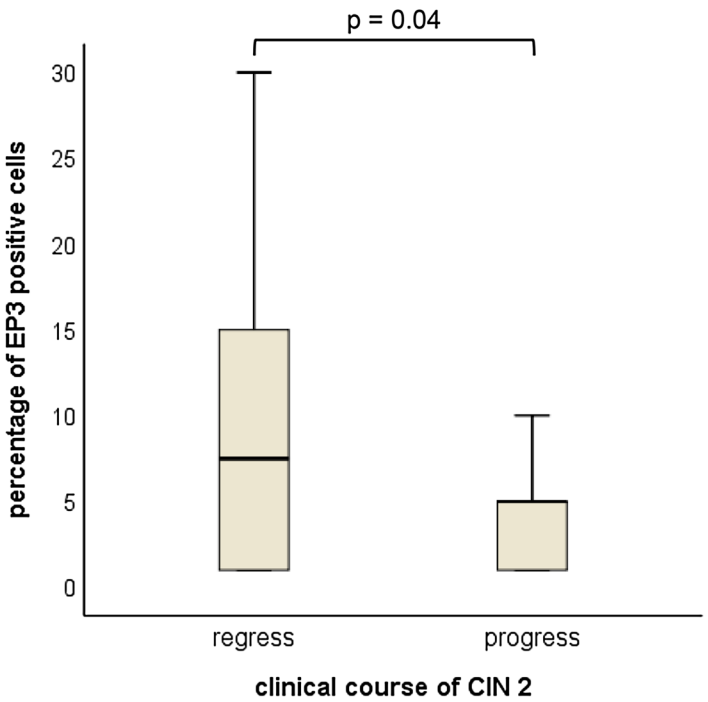
Discussion

The aim of this study was to analyze EP3 receptor expression in patients with cervical dysplasia and to correlate the expression levels with the clinical course in patients with CIN 2 lesions. Interestingly, we were able to demonstrate that all grades of cervical intraepithelial neoplasia show significantly different levels of EP3 expression. Even more, significantly lower numbers of EP3 positive cells were shown in CIN 2 lesions which showed progression compared to regression.

EP3 has been shown to be associated with tumor stage, as well as prognosis in cervical cancer patients (Heidegger et al. 2017). Median IR score of cervical cancer patients was observed to be 2.75, which is in line with our results that show decreasing median IR scores with higher grades of dysplasia (healthy cervical tissue = 12, CIN 1 = 9, CIN 2 = 6, CIN 3 = 4). As in the group of CIN 2 lesions, the percentage of EP3 expressing cells is lower in cases that later show progressive disease, the loss of EP3 expression might be a step towards a higher grade of dysplasia and towards tumorigenesis. However, in cervical cancer patients, higher IRS levels correlated with poor clinical outcome, which could imply that EP3 has different roles in tumorigenesis and progression of established tumors.

The EP3 receptor is one of four identified EP receptors that mediate the effects of prostaglandin E2 (PGE_2). Prostanoids are important mediators in inflammatory and anaphylactic reactions. In addition, they have been shown to promote tumor growth by inhibition of apoptosis, suppression of immune function, and promotion of angiogenesis (Amano et al. 2003; Wang and Dubois 2010). Consequently, prostaglandin and its receptors are increasingly investigated in the context of different malignancies including cervical cancer (Muller 2004; Sales et al. 2001). Prostaglandins are metabolites of arachidonic acid synthesized by the enzyme cyclooxygenase (COX). Two isoforms of this enzyme exist: COX-1 is expressed constitutively in various tissues, whereas COX-2 is only transiently upregulated in inflammation and various tumors like pancreatic, lung, bladder, colon, or prostate cancer (Tsujii et al. 1997; Wang and Dubois 2010).

COX-2 inhibitors like celecoxib have been evaluated in clinical trials for prevention and treatment of tumors (Bertagnolli et al. 2006; Harris 2009). So far, three clinical trials using COX-2 inhibitors have been published in patients with cervical intraepithelial neoplasia. Whereas two of them showed promising results in terms



n	20	18
mean % EP3 positive cells	9,3 %	3,8 %

Fig. 3 Percentage of EP3 positive cells in CIN 2 lesions with progress or regress. Boxplots of percentages of EP3 positive cells in CIN 2 lesions with histologically confirmed regressive (left) or progressive (right) disease are shown. Number of samples analyzed in each group (*n*) and mean percentage of EP3 positive cells are displayed in the table below the graph

of increased regression rates of high-grade CIN lesions compared to the placebo arm, the third study was not able to confirm these effects (Farley et al. 2006; Hefler et al. 2006; Rader et al. 2017). According to the authors, lack of effectiveness in this study might have been attributed to the fact that a lower dose of celecoxib was applied than had been originally planned. This was due to safety concerns as it had been observed that selective COX-2 inhibitors caused rare, but serious cardiovascular complications (Rader et al. 2017; Strand 2007).

Targeting prostaglandin receptors like EP3 might represent a promising alternative option for chemoprevention and treatment as the EP3 receptor has been shown to be associated with carcinogenesis, malignant aggressiveness, and poor outcome in several cancer types (Semmlinger et al. 2018; Yano et al. 2002). However, there are also studies that demonstrate anti-tumorigenic effects of EP3 receptor upregulation (Huang et al. 2013; Kashiwagi et al. 2013; Shoji et al. 2004). These discrepancies might be attributed to the existence of various EP3 isoforms but need to be explored further, before EP3 targeting can be successfully applied in cancer therapy.

In conclusion, we could show that EP3 expression is significantly different in all grades of cervical intraepithelial neoplasia, which suggests that EP3 plays a relevant role in carcinogenesis of cervical cancer. The amount of EP3 expressing cells in CIN 2 lesions seems to be a marker for progression or regression of CIN 2. Therefore, EP3 expression might be an additional factor to help in the decision process regarding the eligibility for non-surgical treatment of a patient with a CIN 2 lesion. Conservative management of selected CIN 2 lesions would help preventing over-therapy and the pregnancy-associated complications of conisation in patients of childbearing-age.

Funding This study was financially supported by Heuer-Foundation.

Compliance with ethical standards

Conflict of interest A. H. has received research Grants from the "Walter Schulz Stiftung" and a speaker and advisory board honorarium from Roche, Germany. T. M. K. is employed at Roche at the time of manuscript submission. S. M. received research support, advisory board, honoraria and travel expenses from AstraZeneca, Clovis, Medac, MSD, PharmaMar, Roche, Sensor Kinesis, Tesaro and Teva. All other authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The current study was approved by the Ethics Committee of the Ludwig-Maximilians-University, Munich, Germany (167-14).

Informed consent Informed consent was obtained from all individual participants included in the study.

Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

References

Amano H et al (2003) Host prostaglandin E2-EP3 signaling regulates tumor-associated angiogenesis and tumor growth. J Exp Med 197:221–232

Arbyn M et al (2008) Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: meta-analysis. BMJ 337:a1284. https://doi.org/10.1136/bmj.a1284337/sep18_1/a1284

Bertagnolli MM et al (2006) Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med 355:873–884. https://doi.org/10.1056/NEJMoa061355

Brummer O, Hollwitz B, Bohmer G, Kuhnle H, Petry KU (2006) Human papillomavirus-type persistence patterns predict the clinical outcome of cervical intraepithelial neoplasia. Gynecol Oncol 102:517–522. https://doi.org/10.1016/j.ygyno.2006.01.020

Cox JT, Schiffman M, Solomon D, Group A-LTS (2003) Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy. Am J Obstet Gynecol 188:1406–1412

Darragh TM et al (2012) The lower anogenital squamous terminology standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Arch Pathol Lab Med 136:1266–1297. https://doi.org/10.5858/arpa.LGT200570

Farley JH, Truong V, Goo E, Uyehara C, Belnap C, Larsen WI (2006) A randomized double-blind placebo-controlled phase II trial of the cyclooxygenase-2 inhibitor celecoxib in the treatment of cervical dysplasia. Gynecol Oncol 103:425–430. https://doi.org/10.1016/j.ygyno.2006.03.036

Harris RE (2009) Cyclooxygenase-2 (cox-2) blockade in the chemoprevention of cancers of the colon, breast, prostate and lung. Inflammopharmacology 17:55–67. https://doi.org/10.1007/s10787-009-8049-8

Hefler LA, Grimm C, Speiser P, Sliutz G, Reinthaller A (2006) The cyclooxygenase-2 inhibitor rofecoxib (Vioxx) in the treatment of cervical dysplasia grade II–III: a phase II trial. Eur J Obstet Gynecol Reprod Biol 125:251–254. https://doi.org/10.1016/j.ejogrb.2005.08.019

Heidegger H et al (2017) The prostaglandin EP3 receptor is an independent negative prognostic factor for cervical cancer patients. Int J Mol Sci. https://doi.org/10.3390/ijms18071571

Huang HF, Shu P, Murphy TF, Aisner S, Fitzhugh VA, Jordan ML (2013) Significance of divergent expression of prostaglandin EP4 and EP3 receptors in human prostate cancer. Mol Cancer Res 11:427–439. https://doi.org/10.1158/1541-7786.MCR-12-0464

Jin G, LanLan Z, Li C, Dan Z (2014) Pregnancy outcome following loop electrosurgical excision procedure (LEEP) a systematic review and meta-analysis. Arch Gynecol Obstet 289:85–99. https://doi.org/10.1007/s00404-013-2955-0

Jordan J et al (2009) European guidelines for clinical management of abnormal cervical cytology, part 2. Cytopathology 20:5–16. https://doi.org/10.1111/j.1365-2303.2008.00636.x

Kashiwagi E, Shiota M, Yokomizo A, Itsumi M, Inokuchi J, Uchiumi T, Naito S (2013) Prostaglandin receptor EP3 mediates growth inhibitory effect of aspirin through androgen receptor and

contributes to castration resistance in prostate cancer cells. Endocr Relat Cancer 20:431–441. https://doi.org/10.1530/ERC-12-0344

Kolben TM et al (2017) Expression of Sialyl Lewis a, Sialyl Lewis x, Lewis y, Gal-3, Gal-7, STMN1 and p16 in cervical dysplasia. Future Oncol 13:145–157. https://doi.org/10.2217/fon-2016-0259

Kyrgiou M, Koliopoulos G, Martin-Hirsch P, Arbyn M, Prendiville W, Paraskevaidis E (2006) Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: systematic review and meta-analysis. Lancet 367:489–498. https://doi.org/10.1016/S0140-6736(06)68181-6

Massad LS et al (2013) 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol 121:829–846. https://doi.org/10.1097/AOG.0b013e3182883a34

Moscicki AB et al (2004) Regression of low-grade squamous intraepithelial lesions in young women. Lancet 364:1678–1683. https://doi.org/10.1016/S0140-6736(04)17354-6

Muller R (2004) Crosstalk of oncogenic and prostanoid signaling pathways. J Cancer Res Clin Oncol 130:429–444. https://doi.org/10.1007/s00432-004-0570-y

Rader JS et al (2017) A stratified randomized double-blind phase II trial of celecoxib for treating patients with cervical intraepithelial neoplasia: the potential predictive value of VEGF serum levels: an NRG Oncology/Gynecologic Oncology Group study. Gynecol Oncol 145:291–297. https://doi.org/10.1016/j.ygyno.2017.02.040

Sales KJ et al (2001) Cyclooxygenase-2 expression and prostaglandin E(2) synthesis are up-regulated in carcinomas of the cervix: a possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors. J Clin Endocrinol Metab 86:2243–2249. https://doi.org/10.1210/jcem.86.5.7442

Semmlinger A et al (2018) EP3 (prostaglandin E2 receptor 3) expression is a prognostic factor for progression-free and overall survival in sporadic breast cancer. BMC Cancer 18:431. https://doi.org/10.1186/s12885-018-4286-9

Shoji Y et al (2004) Downregulation of prostaglandin E receptor subtype EP3 during colon cancer development. Gut 53:1151–1158. https://doi.org/10.1136/gut.2003.028787

Strand V (2007) Are COX-2 inhibitors preferable to non-selective non-steroidal anti-inflammatory drugs in patients with risk of cardiovascular events taking low-dose aspirin? Lancet 370:2138–2151. https://doi.org/10.1016/S0140-6736(07)61909-6

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics, 2012. CA Cancer J Clin 65:87–108. https://doi.org/10.3322/caac.21262

Trimble CL et al (2005) Spontaneous regression of high-grade cervical dysplasia: effects of human papillomavirus type and HLA phenotype. Clin Cancer Res 11:4717–4723. https://doi.org/10.1158/1078-0432.CCR-04-2599

Tsujii M, Kawano S, DuBois RN (1997) Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci USA 94:3336–3340

Wang D, Dubois RN (2010) Eicosanoids and cancer. Nat Rev Cancer 10:181–193. https://doi.org/10.1038/nrc2809

Wilkinson TM, Sykes PH, Simcock B, Petrich S (2015) Recurrence of high-grade cervical abnormalities following conservative management of cervical intraepithelial neoplasia grade 2. Am J Obstet Gynecol 212:769. https://doi.org/10.1016/j.ajog.2015.01.010 e761–767

Yano T, Zissel G, Muller-Qernheim J, Jae Shin S, Satoh H, Ichikawa T (2002) Prostaglandin E2 reinforces the activation of Ras signal pathway in lung adenocarcinoma cells via EP3. FEBS Lett 518:154–158