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RESEARCH

Prognostic Significance of Human Papillomavirus (HPV) Status and Expression of Selected Markers (HER2/neu, EGFR, VEGF, CD34, p63, p53 and Ki67/MIB-1) on Outcome After (Chemo-) Radiotherapy in Patients with Squamous Cell Carcinoma of Uterine Cervix

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Abstract The aim of the retrospective study was to evaluate prognostic significance of human papillomavirus (HPV) status and expression of epidermal growth factor receptor (EGFR), human epidermal growth factor receptor type 2 (HER2/neu), vascular endothelial growth factor (VEGF), CD34 antigen, tumor suppressors p63 and p53, and Ki67/MIB-1 in squamous cell carcinoma of the uterine cervix (SCCC) treated with radiotherapy or chemoradiotherapy. Seventy-two consecutive patients with SCCC, diagnosed and treated with (chemo-) radiotherapy with a curative intent at the University Hospital Hradec Kralove between August 1998 and August 2008, were enrolled in the study. The median follow-up period was 57 months (range 5–152). The tested biological factors were evaluated by polymerase chain reaction (HPV status) and by immunohistochemistry (remaining above mentioned markers) from archival paraffin embedded original diagnostic tumor samples. A statistical significant correlation was observed between low expression of p63 and poor overall survival ($p=0.001$), although the complete response probability was influenced with borderline statistical significance ($p=0.05$). However, the results could be affected by the statistical error due to the small number of p63 negative patients. HPV positivity and EGFR staining intensity was associated with higher complete response probability ($p=0.038$ and $p=0.044$, resp.). All other results were not significant. Neither HPV positivity nor EGFR staining intensity were reflected in the overall survival evaluation. In conclusion, the presented study did not confirm any apparently significant association of the suggested markers with prognosis of SCCC in patients treated with (chemo-) radiotherapy.

Keywords Uterine cervix · Immunohistochemistry · Squamous cell carcinoma · Prognostic factors · Human papillomavirus

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Introduction

Radiotherapy is a basic curative approach for locally and regionally advanced cervical cancer. The irradiation is delivered in most cases with combination of external beam radiotherapy (EBRT) and brachytherapy (BRT). Currently, the biological effect of radiotherapy (RT) is standardly enhanced by concurrent administration of cisplatin-based chemotherapy. Despite a relative radiosensitivity of squamous cell cervical carcinoma (SCCC) the treatment results are not optimal, the 5-year overall survival being about 52 % [1].

Total dose of irradiation and total treatment time are important factors for treatment results of (chemo-) radiotherapy. On the contrary, higher doses are associated with increased toxicity of RT. Especially late complications (e.g. rectal bleeding, bowel stenosis, fistula, ulceration or radiation cystitis) have direct influence on quality of life [2] and could be fatal in extreme cases. Therefore, there is an attempt to find predictors of tumor response after standard chemoradiotherapy to individualize the

treatment approach. Many articles demonstrated promising relationships between various imunohistochemically evaluated biomarkers and treatment response or prognosis in cervical cancer patients in recent years. The review article was published recently by Petera et al. [3]. Unfortunately, the effect of expression of individual proteins on treatment outcome is still not certain and unambiguous.

Therefore, we decided to evaluate the influence of expression of selected potential predictors, assessed by immunohistochemistry, on treatment outcomes in a cohort of patients treated at our institution. The clinical results of whole group of patients with cervical cancer treated at our department were referred repeatedly earlier [4–6].

The following proteins were selected for the study: cell-surface receptors—epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor type 2 (HER2/Neu), markers of angiogenesis—vascular endothelial growth factor (VEGF) and CD34 antigen, tumor suppressors p53 and p63, and marker of cell proliferation Ki67/MIB-1. Besides the immunohistochemically evaluated proteins we added the human papillomavirus (HPV) status as a possible prognostic factor.

Material and Methods

Patients

We retrospectively reviewed the treatment results of 72 consecutive patients with histologically confirmed squamous cell carcinoma of the uterine cervix, who received radiotherapy or chemoradiotherapy with curative intent at the University Hospital Hradec Kralove (Hradec Kralove, Czech Republic) between August 1998 and August 2008. The median patient age was 50 years (range, 28–81 years). Tumor stage and treatment characterics are listed in Table 1. The median follow-up period was 57 months (range, 3–153 months).

In all but eleven patients complete remission was reached after the treatment (84.7 %). Thirty two patients (44.4 %) are alive, 26 (36.1 %) patients died of the disease, two patients died of other cancer, 8 patients died of other reasons (in four cases deaths could be associated with late toxicity) and two patients were lost from follow-up. The median survival was 69 months (5.7 years), 5-years overall survival was 52.9 % (95 % CI 41.2–64.6 %).

Table 1 Patient and treatment characteristics. TNM stage according to AJCC/UICC TNM Staging System, 6th edition; 2002 [32]

Characteristic	Number
T-stage	
T1b	1
T2a	2
T2b	50
T3a	0
T3b	18
T4	1
N-stage	
N0	32
N1	40
M-stage	
M0	68
M1 (PALN)	4
RT	
EBRT of pelvis	72
EBRT of PALN	37
Brachytherapy	68
Chemotherapy	
Concurrent cisplatin	46
Concurrent paclitaxel	6
Neoadjuvant chemotherapy	1

RT radiotherapy; *EBRT* external beam radiotherapy; *PALN* paraaortic lymph nodes

Analysis of Potential Predictors

Tissue specimens were immediately fixed in 10 % formalin, routinely processed, embedded in paraffin, and stained with hematoxylin-eosin. The paraffin blocks for HPV testing and immunohistochemical analysis were available in all cases and were retrieved from the archive of the Fingerland Department of Pathology, University Hospital Hradec Králové.

HPV

The HPV DNA detection was performed by polymerase chain reaction (PCR) as follows. The HPV DNA was extracted from paraffin-embedded tissue after deparaffinization in xylen and rehydration in ethanol using the commercial QIAamp DNA FFPE Tissue Kit (Qiagen GmBH, Hilden, Germany) according to the manufacturer's protocol. PCR amplification of β-globin sequences was performed to confirm sample fitness for PCR assay. All samples were screened for presence of HPV DNA by PCR amplification with primers GP5+/GP6+ located within the HPV L1 gene. The sequences of the forward and reverse primers used were 5′-TTTGTTACTGTGGTAGATACTAC-3′ (GP5+) and 5′-GAAAAATAAACTGTAAATCATATT-3′ (GP6+). The PCR reaction was performed in a volume of 25 μL, containing 25 mM MgCl2, 2.5 mM of each dNTP, 2.5 units of Takara Taq polymerase (Takara Bio Inc., Shiga, Japan), 100 pmol of each primer (GP5+/GP6+) and 2 μL of HPV DNA at various dilutions. The PCR protocol was then carried out with an initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 60 s, and extension at 72 °C for 45 s. Amplified products were run on 2 % agarose gel and

stained with ethidium bromide for size verification. Samples showing HPV DNA presence by the above mentioned procedure were subsequently analyzed using Linear Array HPV Genotyping Test (Roche, Basel, Switzerland). The manufacturer's protocol was modified to adapt the test for use on paraffin-embedded tissue according to Siriaunkgul et al. [7]. The test involves three steps: PCR amplification of target DNA, nucleic acid hybridization, and detection of 37 HPV types, specifically 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39 a CP6108. PCR was performed in a total volume of 100 μL containing 50 μL of the manufacturer´s master mix and 50 μL of GP5+/GP6+ PCR product. The amplification program consisted of 2 min at 50 °C, and 9 min at 95 °C, followed by 40 cycles of 30 s at 50 °C, of 1 min at 55 °C, and of 1 min at 72 °C, with a final extension at 72 °C for 5 min. The PCR product was denaturated with denaturation solution and hybridized on to a strip containing specific probes for the 37 above mentioned HPV types and β-globin reference lines. Detection was carried out using streptavidin-HRP and 0.1 % tetramethylbenzidine as a chromogen. Positive reaction was visible as a blue line on the strip.

Immunohistochemistry

Four μm-thick sections were cut from paraffin blocks, mounted on slides coated with 3-aminopropyltriethoxysilane, deparaffinized in xylene, and rehydrated in descending grades (100 % to 70 %) of ethanol. Indirect immunohistochemistry using polyclonal and monoclonal antibodies against Her-2/Neu oncoprotein (HercepTest™ kit), epidermal growth factor receptor (EGFR) (EGFR pharmDx™ kit), vascular endothelial growth factor (VEGF) (clone VG1, dilution 1:200), CD34 (QBEnd 10, 1:50), p63 protein (4A4, 1:200), p53 protein (DO-7, 1:300) and Ki-67 (MIB-1, 1:50) was performed. The source of both detection kits and of all antibodies was Dako (Dako Denmark A/S, Glostrup, Denmark). The staining of all markers was done manually. The detection of both Her-2/neu oncoprotein and EGFR was performed according to the instruction of the manufacturer (Dako). Antigen retrieval was performed in a water bath for 40 min at 97 °C in the retrieval buffer S2367 (Dako) at pH 9.0 for CD34, p53 and Ki-67. For VEGF and p63, the tissue was processed in the microwave vacuum histoprocessor RHS 1(Milestone Srl, Sorisole, Italy) at pH 6.0 at 120 °C for 4 min. Endogenous peroxidase activity was inhibited by immersing the sections in 3 % hydrogene peroxide. Finally, the sections were incubated with EnVision™ Dual Link System-HRP (Dako) and the reaction was visualized using diaminobenzidine. Then, the slides were counterstained with hematoxylin. Appropriate positive and negative controls were used. The evaluation criteria are summarized in Tables 2 and 3. The study slides (and corresponding controls) were read independently by two study pathologists (J.L. and E.H.). Any discrepant cases were resolved by consensus review. The expression of Her-2/neu was assessed according to the HER-score system. The CD34 expression was used for identification of endothelium of new-formed vessels in so-called "hot spots", The number of new-formed vessels in five power fields (x10 eyepiece and x20 lens; area 1.23 mm^2) was counted and the average number of new-formed vessels per one power field was recorded (Gaffney et al.). The expression of all remaining markers was scored in 10 % increments as percentage of positive tumor cells. The expression of each marker was evaluated in three high power fields (HPFs) (x10 eyepiece and x40 lens; area 0.15 mm^2) in the tumor area where the highest expression was detected at low magnification (x10 eyepiece and x4 lens; area 30.18 mm^2). In each HPF, one hundred tumor cells were assessed and the number of positive cells was recorded. Finally, an average was calculated from these three values and rounded to the tens. The threshold of positivity was defined as staining of more than 10 % of tumor cells. Staining intensity of EGFR was assessed according to the manufacturer's interpretation manual (Dako) and of p53 and VEGF it was interpreted as weak (=1), moderate (=2) or strong (=3). If heterogenous staining intensity was observed, the most intensive degree was

Table 2 Evaluation criteria

Marker	Staining type	Evaluation
Her-2/neu oncoprotein[a]	Membranous	HER-score system: (0), (1+), (2+), (3+)
EGFR[b]	Membranous	Percent of stained tumor cells (with 10 % accuracy) and staining intensity (1+, 2+, 3+)
VEGF	Cytoplasmic	Percent of stained tumor cells (with 10 % accuracy) and staining intensity (mild, moderate, strong)
CD34	Membranous	Average number of new-formed vessels in 1 power field (PF) as calculated from 5 PFs, analyzed using 20x lens and 10x eyepiece (area 1.23 mm^2)
p63	Nuclear	Percent of stained tumor cells (with 10 % accuracy)
p53	Nuclear	Percent of stained tumor cells (with 10 % accuracy) and staining intensity (mild, moderate, strong)
Ki-67	Nuclear	Percent of stained tumor cells (with 10 % accuracy)

[a] Evaluation system adopted from HercepTest™ Interpretation Manual (Dako)

[b] Evaluation system adopted from EGFR pharmDx™ Interpretation Manual (Dako)

Table 3 Comment to analyzed results of individual evaluated biological factors

Factor	Result
HPV	HPV positivity was demonstrated in 57 samples (79 %). The samples were in a sufficient quality for subsequent HPV genotype testing in 45 cases. Type 16 was found in 34 cases (75 %), type 18 in 5 cases (11 %), other catched types were 35 (1 case), 42 (1 case), 45 (3 cases) and 59 (1 case).
Her2/Neu	68 of 70 samples (98 %) were HER2/Neu negative (HER2 score 0 or 1+), only two cases were 2+ and no case was 3+.
EGFR	The median value of percentage of stained cells was 35 % (0–100 %). The staining intensity of grade 0, 1, 2 and 3 was in 10, 6, 24 and 30 cases, respectively
VEGF	The median value of percentage of stained cells was 100 % (40–100 %). The staining intensity of grade 0, 1, 2 and 3 was in 0, 4, 34 and 32 cases, respectively
CD34	The median value of average number of new-formed vessels in 1 power field was 64.5 (40–210).
p63	64 of 70 samples were highly positive (80–100 %), in 40 cases 100 %. Two samples were moderately positive (both 60 %) and four were negative (0–10 %) in p63 staining.
p53	The median value of percentage of stained cells was 22.5 % (0–90 %). The staining intensity of grade 0, 1, 2 and 3 was in 1, 13, 34 and 22 cases, respectively.
Ki67/ MIB-1	The median value of Ki67 stained cells was 80 % (range 30–100 %).

recorded. The staining intensity of p63 and Ki-67 was not qualified as it was strong in all cases.

Statistical Methods

All potential biological factors were correlated with presence of complete response and overall survival. We decided not to primary correlate the analyzed factors with local control or freedom of the disease due to uncertain information in few cases of death outside our institution.

Basic descriptive statistics were adopted for the analysis: median and 95 % confidence interval for continuous data, and absolute and relative frequencies for categorical data. Kaplan-Maier and Logrank tests were used for survival analysis. Univariate and multivariate Cox regression analysis was used to determine the influence of patient/tumor characteristics and immunohistochemical markers upon survival. Logistic regression was adopted to analyse the relationship between patient characteristics or immunohistochemical markers and the treatment response. Relationship between immunohistochemical markers and treatment response was also analyzed using the chi-square test; while Fisher's exact test was used in a four-field table when the number of cases was fewer than 10. We considered $p<0.05$ to be statistically significant. All statistical analyses were performed using the NCSS 8 statistical software program (NCSS, Keysville, Utah).

Results

Tumor samples of all 72 patients were analyzed for HPV analysis. Samples of 70 patients were subsequently analyzed for immunohistochemical methods. In two cases there was not sufficient material for immunohistochemical analysis.

Relationships of analyzed HPV positivity and protein expressions and response to treatment and overall survival are presented in Tables 4 and 5, respectively. A statistical significant association was observed between low expression of p63 and poor overall survival (both univariate and multivariate analysis $p=0.001$), although the complete response probability was influenced with borderline statistical significance ($p=0.05$).

HPV positivity and high EGFR intensity was associated with higher complete response probability ($p=0.038$ and $p=0.044$, resp.). The influence of HPV positivity on complete response probability was confirmed by chi-square test ($p=0.029$) and Fisher's exact test ($p=0.044$). The association of EGFR intensity and complete response probability was not confirmed by statistical significant result using chi-square test ($p=0.087$).

All other results were not significant. Neither HPV positivity nor high EGFR intensity were reflected in the overall survival evaluation.

Discussion

The correlation of HPV positivity with the prognosis of cervical cancer patients is very controversial. Some studies

Table 4 Relationship of analyzed factors and treatment response using logistic regression analysis

Factor	Probability level	Odds ratio (95 % confidence interval)
HPV positivity	0.038	4.25 (1.08–16.67)
HER score	0.41	2.28 (0.31–16.52)
EFGR %	0.70	1.00 (0.98–1.02)
EGFR intensity	0.044	1.81 (1.01–3.25)
VEGF %	0.32	0.96 (0.88–1.04)
VEGF intensity	0.19	2.03 (0.70–5.94)
CD34	0.78	1.00 (0.97–1.04)
P63 %	0.050	1.02 (1.00–1.04)
P53 %	0.69	0.99 (0.96–1.03)
P53 intensity	0.96	1.02 (0.48–2.43)
Ki67 %	0.55	1.01 (0.97–1.05)

Table 5 Relationship of analyzed factors and overall survival using univariate Cox-regression analysis

Factor	Probability level	Risk ratio (95 % confidence interval)
HPV positivity	0.31	0.67 (0.30–1.46)
HER score	0.77	1.10 (0.58–2.06)
EFGR %	0.40	0.996 (0.987–1.005)
EGFR intensity	0.19	0.82 (0.61–1.10)
VEGF %	0.23	1.02 (0.99–1.05)
VEGF intensity	0.17	0.70 (0.42–1.17)
CD34	0.88	0.999 (0.985–1.013)
P63 %	0.001	0.98 (0.97–0.99)
P53 %	0.43	0.99 (0.98–1.01)
P53 intensity	0.97	1.01 (0.65–1.56)
Ki67 %	0.07	0.98 (0.96–1.00)

confirmed poorer prognosis of HPV negative cervical cancer patients. Harima et al. demonstrated the HPV positivity in 76.2 % of patients. The HPV positivity was associated with better disease-free survival and overall survival [8]. Similar results were published by Lindel et al. In this study the ratio of HPV positive was 70 % and there was statistically significant better complete response rate, local control, disease free survival and overall survival [9]. Conversely, according to other authors the HPV negativity of cervical cancer samples means only the insufficiency of HPV diagnostics [10]. HPV positivity in the presented study samples was clearly demonstrated in 79.1 %. There was found a statistically significant association between HPV negativity and worse response; otherwise no correlation with overall survival was captured. A possible explanation for this observation could be a higher number of HPV negative patients in older age group who were treated with radiotherapy only with worse complete response probability. But the possible association of higher age of patients and HPV negativity is uncertain.

The published results of expression of HER2/Neu as a prognostic marker are divergent. In the study of Lee et al. the increased HER2/Neu expression was associated with improved overall survival [11]. Conversely, in other studies HER2/neu overexpression was negative prognostic factor [12, 13]. In the presented study HER2/Neu 3+ was not found in any case, and only two cases were evaluated as 2+. Therefore, we cannot confirm, HER2/Neu overexpression might be a prognostic marker. Our results are similar to results of Shen et al. (no HER2 positive sample of 53 analyzed cases) [14]. The advantage of the presented study was the use of certified kit.

Previous data suggest that EGFR overexpression is associated with poorer overall survival and disease free survival [15, 16]. No correlation with prognosis was found in EGFR expression evaluation in other studies [12, 14]. Presented study does not confirm the influence of EGFR overexpression on overall survival. Furthermore, the logistic regression analysis has shown (although borderline) the opposite effect of EGFR expression on response to (chemo-)radiotherapy – higher EGFR intensity was more frequently demonstrated in patients with complete response ($p=0.044$).

In the trial of Loncaster et al. high VEGF expression was associated with a poor prognosis [17]. VEGF was the most significant independent prognostic factor for overall survival, metastasis free survival, but not for local control after radiotherapy. Similarly, relationship between overexpression of VEGF and poor prognosis was found in other studies [18, 19]. On the contrary, these results were not confirmed by Gynecologic Oncology Group study [20]. Our results do not confirm these results as well. We have not found any correlation between VEGF expression and overall survival or complete response.

There is only one study testing an influence of marker of microvessel density CD34 on the prognosis of SCCC patients treated by with radiotherapy. The number of newformed vessels, which were highlighted by CD34 immunostaining of endothelium, did not correlate with disease free survival or overall survival [19]. Our results are fully consistent with this study.

Tumor protein p63 is a member of the p53 family of transcription factors. In the study of Cho et al. the p63 expression (IIB stage SCCC) was associated with worse overall survival and locoregional failure [16]. The presented study does not confirm this result. Quite the contrary, our statistical result is that low expression of p63 is associated with poor results in term of overall survival and complete response probability. The reason for this fact can be high number of p63 positive samples (91 %) and poor treatment results in remaining six patients in our study. The number of p63 negative patients is too small for conclusion.

The correlation of p53 with prognosis in SCCC is controversial. Oka et al. found p53 to be a marker of poor prognosis [21]. In the trail of Jain, the p53 expression correlated with poor prognosis in univariate analysis, but not upon multivariate analysis [22]. On the contrary, Lindstrom et al. found higher 10-years overall survival in patients with p53 expression in multivariate analysis (but not in Cox regression analyses) [23]. Other studies, including the presented study, found no influence on the prognosis of patients with SCCC [12, 24–26].

Low Ki67 expression was associated with poor prognosis due to local recurrence following radiation therapy in study of Nakano et al. [27], and similarly, higher expression of Ki67 was positive prognostic factor in other studies [28, 29]. On the contrary, other trials found no correlation [12, 30, 31]. The median value of Ki67 stained cells was higher than in mentioned studies above. Ki67 expression had no influence on complete response probability, but there was captured a

trend to better overall survival in univariate Cox regression analysis in Ki67 high expression ($p=0.07$).

Conclusion

The results of presented study do not confirm clear significance of any of previously suggested immunohistochemical prognostic factor in SCCC in patients treated with (chemo-) radiotherapy in terms of overall survival. Higher complete response probability in HPV positive tumors is controversial. In our opinion, further research on possible prognostic factors in SCCC should be conducted on the basis of molecular biology methods.

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References

1. Green JA, Kirwan JM, Tierney JF, Symonds P, Fresco L, Collingwood M, Williams CJ (2001) Survival and recurrence after concomitant chemotherapy and radiotherapy for cancer of the uterine cervix: a systematic review and meta-analysis. Lancet 358:781–786
2. Gondi V, Bentzen SM, Sklenar KL, Dunn EF, Petereit DG, Tannehill SP, Straub M, Bradley KA (2012) Severe late toxicities following concomitant chemoradiotherapy compared to radiotherapy alone incervical cancer: an inter-era analysis. Int J Radiat Oncol Biol Phys 84:973–982. doi:10.1016/j.ijrobp.2012.01.064
3. Petera J, Sirak I, Beranek M, Vosmik M, Drastikova M, Paulikova S, Soumarova R (2011) Molecular predictive factors of outcome of radiotherapy in cervical cancer. Neoplasma 58:469–475
4. Sirák I, Petera J, Odrážka K, Doležel M, Zoul Z, Vaculíková M (2006) Chemotherapy intensity importance in concurrent chemoradiotherapy of locally advanced cervical cancer. Ceska Gynekol 71:308–311
5. Petera J, Odrážka K, Doležel M, Zoul Z, Vaculíková M, Šefrová J, Sirák I (2007) Extended field radiotherapy and high-dose brachytherapy combined with chemotherapy in patients with locally advanced cervical carcinoma. Ceska Gynekol 72:126–130
6. Sirák I, Petera J, Zoul Z (2008) The influence of cisplatin dose upon survival in concurrent chemoradiotherapy of locally advanced cervical carcinoma with weekly cisplatin. Acta Medica (Hradec Kralove) 51:95–99
7. Siriaunkgul S, Suwiwat S, Settakorn J, Khunamornpong S, Tungsinmunkong K, Boonthum A, Chaisuksunt V, Lekawanvijit S, Srisomboon J, Thorner PS (2008) HPV genotyping in cervical cancer in Northern Thailand: adapting the linear array HPV assay for use on paraffin-embedded tissue. Gynecol Oncol 108:555–560. doi:10.1016/j.ygyno.2007.11.016
8. Harima Y, Sawada S, Nagata K, Sougawa M, Ohnishi T (2002) Human papilloma virus (HPV) DNA associated with prognosis of cervical cancer after radiotherapy. Int J Radiat Oncol Biol Phys 52:1345–1351
9. Lindel K, Burri P, Studer HU, Altermatt HJ, Greiner RH, Gruber G (2005) Human papillomavirus status in advanced cervical cancer: predictive and prognostic significance for curative radiation treatment. Int J Gynecol Cancer 15:278–284
10. Herrington CS (1999) Do HPV-negative cervical carcinomas exist?–revisited. J Pathol 189:1–3
11. Lee CM, Shrieve DC, Zempolich KA, Lee RJ, Hammond E, Handrahan DL, Gaffney DK (2005) Correlation between human epidermal growth factor receptor family (EGFR, HER2, HER3, HER4), phosphorylated Akt (P-Akt), and clinical outcomes after radiation therapy in carcinoma of the cervix. Gynecol Oncol 99:415–421
12. Yamashita H, Murakami N, Asari T, Okuma K, Ohtomo K, Nakagawa K (2008) Correlation among six biologic factors (p53, p21(WAF1), MIB-1, EGFR, HER2, and Bcl-2) and clinical outcomes after curative chemoradiation therapy in squamous cell cervical cancer. Int J Radiat Oncol Biol Phys 74:1165–1172. doi:10.1016/j.ijrobp.2008.09.005
13. Niibe Y, Nakano T, Ohno T, Suzuki Y, Oka K, Tsujii H (2003) Prognostic significance of c-erbB-2/HER2 expression in advanced uterine cervical carcinoma with para-aortic lymph node metastasis treated with radiation therapy. Int J Gynecol Cancer 13:849–855
14. Shen L, Shui Y, Wang X, Sheng L, Yang Z, Xue D, Wei Q (2008) EGFR and HER2 expression in primary cervical cancers and corresponding lymph node metastases: implications for targeted radiotherapy. BMC Cancer 8:232. doi:10.1186/1471-2407-8-232
15. Noordhuis MG, Eijsink JJ, Ten Hoor KA, Roossink F, Hollema H, Arts HJ, Pras E, Maduro JH, Reyners AK, de Bock GH, Wisman GB, Schuuring E, van der Zee AG (2009) Expression of epidermal growth factor receptor (EGFR) and activated EGFR predict poor response to (chemo)radiation and survival in cervical cancer. Clin Cancer Res 15:7389–7397. doi:10.1158/1078-0432.CCR-09-1149
16. Cho NH, Kim YB, Park TK, Kim GE, Park K, Song KJ (2003) P63 and EGFR as prognostic predictors in stage IIB radiation-treated cervical squamous cell carcinoma. Gynecol Oncol 91:346–353
17. Loncaster JA, Cooper RA, Logue JP, Davidson SE, Hunter RD, West CM (2000) Vascular endothelial growth factor (VEGF) expression is a prognostic factor for radiotherapy outcome in advanced carcinoma of the cervix. Br J Cancer 83:620–625
18. Kang JO, Hong SE (2004) The prognostic effect of VEGF expression in squamous cell carcinoma of the cervix treated with radiation therapy alone. J Korean Med Sci 19:693–697
19. Gaffney DK, Haslam D, Tsodikov A, Hammond E, Seaman J, Holden J, Lee RJ, Zempolich K, Dodson M (2003) Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) negatively affect overall survival in carcinoma of the cervix treated with radiotherapy. Int J Radiat Oncol Biol Phys 56:922–928
20. Randall LM, Monk BJ, Darcy KM, Tian C, Burger RA, Liao SY, Peters WA, Stock RJ, Fruehauf JP (2009) Markers of angiogenesis in high-risk, early-stage cervical cancer: a Gynecologic Oncology Group study. Gynecol Oncol 112:583–589. doi:10.1016/j.ygyno.2008.11.013
21. Oka K, Suzuki Y, Nakano T (2000) Expression of p27 and p53 in cervical squamous cell carcinoma patients treated with radiotherapy alone: radiotherapeutic effect and prognosis. Cancer 88:2766–2773
22. Jain D, Srinivasan R, Patel FD, Kumari Gupta S (2003) Evaluation of p53 and Bcl-2 expression as prognostic markers in invasive cervical carcinoma stage IIb/III patients treated by radiotherapy. Gynecol Oncol 88:22–28
23. Lindström AK, Stendahl U, Tot T, Lidström BM, Hellberg D (2007) Predicting the outcome of squamous cell carcinoma of the uterine cervix using combinations of individual tumor marker expressions. Anticancer Res 27:1609–1615
24. Ebara T, Mitsuhashi N, Saito Y, Sakurai H, Hasegawa M, Takahashi M, Takahashi T, Hayakawa K, Niibe H (1996) Prognostic significance of immunohistochemically detected p53 protein expression

in stage IIIB squamous cell carcinoma of the uterine cervix treated with radiation therapy alone. Gynecol Oncol 63:216–218
25. Ishikawa H, Mitsuhashi N, Sakurai H, Maebayashi K, Niibe H (2001) The effects of p53 status and human papillomavirus infection on the clinical outcome of patients with stage IIIB cervical carcinoma treated with radiation therapy alone. Cancer 91:80–89
26. Wootipoom V, Lekhyananda N, Phungrassami T, Boonyaphiphat P, Thongsuksai P (2004) Prognostic significance of Bax, Bcl-2, and p53 expressions in cervical squamous cell carcinoma treated by radiotherapy. Gynecol Oncol 94:636–642
27. Nakano T, Oka K, Ishikawa A, Morita S (1998) Immunohistochemical prediction of radiation response and local control in radiation therapy for cervical cancer. Cancer Detect Prev 22:120–128
28. Pillai MR, Jayaprakash PG, Nair MK (1998) Tumour-proliferative fraction and growth factor expression as markers of tumour response to radiotherapy in cancer of the uterine cervix. J Cancer Res Clin Oncol 124:456–461
29. Suzuki M, Tsukagoshi S, Saga Y, Ohwada M, Sato I (2000) Assessment of proliferation index with MIB-1 as a prognostic factor in radiation therapy for cervical cancer. Gynecol Oncol 79:300–304
30. Oka K, Arai T (1996) MIB1 growth fraction is not related to prognosis in cervical squamous cell carcinoma treated with radiotherapy. Int J Gynecol Pathol 15:23–27
31. Levine EL, Renehan A, Gossiel R, Davidson SE, Roberts SA, Chadwick C, Wilks DP, Potten CS, Hendry JH, Hunter RD et al (1995) Apoptosis, intrinsic radiosensitivity and prediction of radiotherapy response in cervical carcinoma. Radiother Oncol 37:1–9
32. Sobin LH, Wittekind C (2002) UICC international union against cancer TNM classification of malignant tumours, 6th edn. Wiley-Liss, New York