Differences in Expression of Tumor Markers Between Pre- and Postmenopausal Women with Invasive Cervical Cancer

DAN HELLBERG[1,2], TIBOR TOT[3] and ULF STENDAHL[4]

[1]*Center for Clinical Research, Falun;*
[2]*Department for Women's and Children's Health, Uppsala University, Uppsala;*
[3]*Department of Pathology and Clinical Cytology, Falun Hospital, Falun;*
[4]*Department of Radiation Sciences, University Hospital, Umeå, Sweden*

Abstract. *The aim of the present study was to investigate the differences in the expression of tumor markers in squamous cell and in adenomatous carcinomas in pre- and postmenopausal women, respectively. Patients and Methods: The study population comprised 53 premenopausal and 107 postmenopausal women. Thirty-four tumors were adenomatous (n=31) or adenosquamous carcinomas (n=3), distributed between 13 premenopausal and 21 postmenopausal women. The remaining 126 squamous cell carcinomas were diagnosed in 40 pre- and 86 postmenopausal women. Expression of ten tumor markers of possible clinical importance in cervical cancer was evaluated. Results: Expression of three tumor markers, p53 (>0% vs. 0%), p27 (≥20% vs. <20%) and cyclooxygenase-2 (COX-2) (high intensity vs. moderate/none) differed significantly between pre- compared to postmenopausal women with squamous cell (p27; 54% vs. 72%, p=0.009) or adenomatous carcinomas (p53; 8% vs. 63%, p=0.006 and COX-2; 46% vs. 20%, p=0.03). All results were adjusted for clinical cancer stage. Conclusion: The unusual age-specific incidence curve in cervical cancer has rarely been related to expression of tumor markers. Age-related differences in expression of tumor markers could reflect some age-related different biological mechanisms in cervical cancer.*

The age-specific incidence curve in cervical cancer is unusual but has barely been investigated biologically. The curve is similar in all geographical areas of the world with a peak occurring between 40 to 55 years of age, followed by a decline and plateau which in turn will often be followed by a slight increase at older ages (1). This could suggest that, despite all similarities in histology, clinical staging, stage-related prognosis *etc.*, there are some different mechanisms between cervical cancer appearing in women of young and old ages. Comparison of the expression of tumor markers in cancer from pre- and postmenopausal women could provide further information on this issue.

Correspondence to: Dan Hellberg, Center for Clinical Research, Nissers väg 3, 79182 Falun, Sweden. Tel: +46 23 492000, Fax: +46 23 18375, e-mail: dan.hellberg@ltdalarna.se

Key Words: Cervical cancer, markers, p53, p27, COX-2, Ki-67, EGFR, E-cadherin, c-myc, VEGF, CD44, CD4, age-specific expression.

Patients and Methods

The women were admitted to the Department of Gynecological Oncology, Umeå, Sweden, during 1984 to 1990. Clinical staging was made according to FIGO (2) and clinicopathological details were recorded. All women were treated with radiotherapy and 44 had surgery. Treatment was in accordance with contemporary routines. The women were followed-up for at least ten years. Immunohistochemical staining was carried out with standard methods and the evaluation of expression was made by a pathologist blinded for clinical details. The material has been presented in detail elsewhere (3). Expression of c-myc (malignant transformation), Ki-67 and epidermal growth factor receptor (EGFR; proliferation), p53, p27 (cell cycle arrest), E-cadherin and CD44 (cell–cell adhesion), vascular endothelial growth factor (VEGF; angiogenesis), cyclooxygenase-2 (COX-2; prostaglandin synthesis), and CD4 (immune response) were assessed.

Results

Age distribution at diagnosis of cervical cancer during the study period is given in Figure 1. The study population comprised 53 premenopausal and 107 postmenopausal women. The mean age of these women was 39.1 years and 68.7 years, respectively. Thirty-four tumors were adenomatous (n=31) or adenosquamous carcinomas (n=3), distributed between 13 premenopausal and 21 postmenopausal women. The remaining 126 tumors were squamous cell carcinomas, distributed in 40 premenopausal and 86 postmenopausal women. There was no significant difference between pre- and postmenopausal women regarding the histological distribution

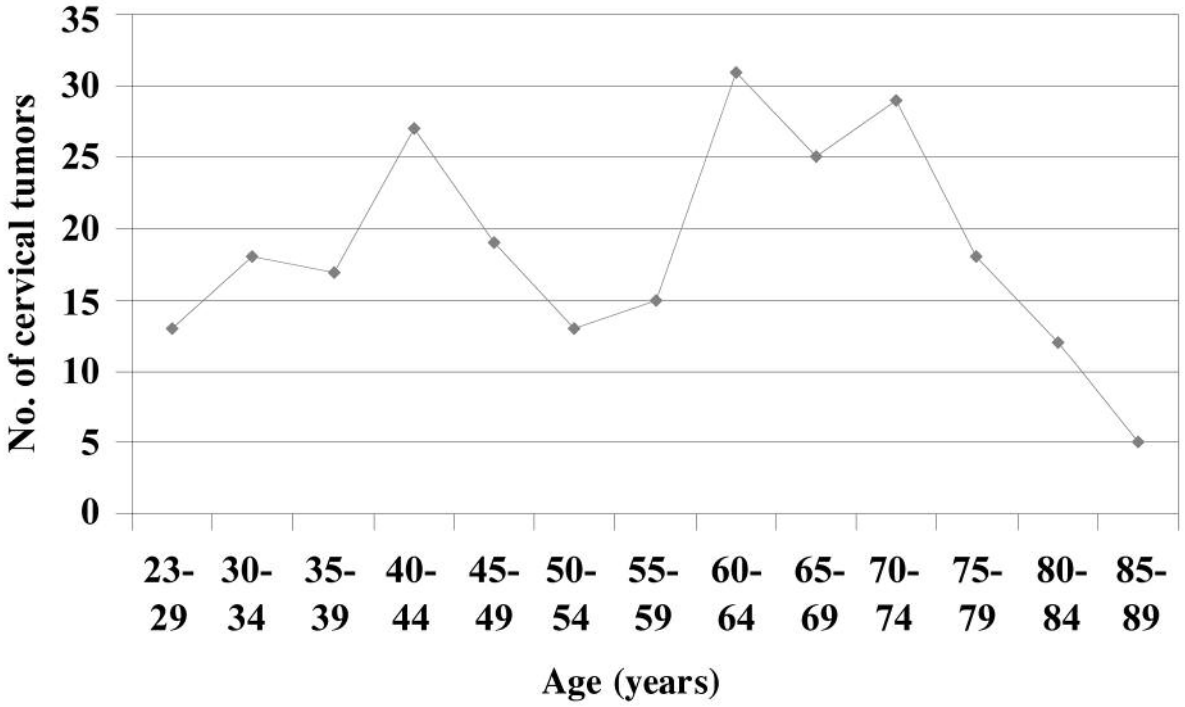

Figure 1. *Age distribution for all cervical cancer diagnosed at the Department of Gynaecological Oncology during 1984-1990.*

of the tumors. Compared to the premenopausal women, the postmenopausal women on average had their cancer diagnosis at a later stage, with 33% evaluated at clinical stage III or IV compared to 8% in the younger group (p=0.0001). There was no difference in cancer-specific 10-year survival after adjustment for stage. Differences in expression of tumor markers between pre- and postmenopausal women distributed among adenosquamous and squamous cell carcinoma are given in Table I. Expression of p27 in squamous cell carcinomas was significantly higher in tumors from post- compared to premenopausal women. In adenomatous/adenosquamous tumors, COX-2 intensity was significantly higher in tumors of premenopausal women, while expression of p53 was higher in postmenopausal women. Expression of these tumor markers in the two groups of women did not correlate with 10-year survival. There were no significant differences in expression of the remaining seven tumor markers that were evaluated between the two groups of women studied. Serum progesterone and estradiol were analysed in 109 women. There were no significant correlations between progesterone and estradiol values and expression of p53, p27 and COX-2.

Discussion

This study provides evidence of some different biological behaviors between pre- and postmenopausal cervical carcinoma as expression of three tumor markers differs. The underlying mechanisms for these discrepancies cannot be explained in this investigation. We are also unable to make comparisons, as to our knowledge the findings are novel. The lower incidence of cervical human papillomavirus (HPV) infections in elderly women, with a sharp decrease between 25 to 35 years of age (4) might lead to different mechanisms in carcinogenesis between the two groups of women in this study. Given an average latency from the initial cervical HPV infection of 10-20 years or even more, the peak age-specific incidence rate of invasive cancer would be explained. HPV DNA is also found at a lower frequency in elderly than in younger women in both squamous cell cervical tumors (5) and adenocarcinomas (6). This could provide an explanation for our findings that there are some differences between pre- and postmenopausal cervical cancer. In the whole study population, high COX-2 intensity and low expression of p53 correlated with a poor prognosis in squamous epithelial cancer after adjustment for clinical stage (3). No significant correlations with survival between p27, p53 and COX-2 and the four subgroups in the present study were found. The relatively small study population with adenomatous/ adenosquamous carcinomas would require large differences in survival rates to reach statistical significance and this is also true for the two subgroups of squamous cell carcinoma analysed here. The p53 tumor suppressor protein causes cell cycle arrest by blocking the cell at the G0/G1 phase and at G2 phase prior to DNA replication and mitosis, respectively, and thereby aid the DNA repair process. p53 is one of the

Table I. *Distribution of tumor markers in pre- and postmenopausal women.*

	Menopausal status		
	Pre n=53	Post n=107	*p*-value[1]
Squamous cell carcinoma			
p27≥20% *vs.* <20%	21 (53.9)	61 (71.8)	0.009
Cox-2 intensity: high *vs.* none/moderate	11 (27.5)	12 (14.0)	0.138
p53>0% *vs.* 0%	25 (61.0)	51 (59.3)	0.588
Adenosquamous carcinoma			
p27≥20% *vs.* <20%	11 (84.6)	14 (66.7)	0.284
Cox-2 intensity: high *vs.* none/moderate	6 (46.2)	4 (20.0)	0.034
p53>0% *vs.* 0%	1 (7.7)	12 (63.2)	0.006

[1]Adjusted for clinical stage IB-IIA *vs.* IIB-IV.

most widely studied tumor markers and the variation in results in different studies is much greater compared to studies of other tumor markers. Some reports correlate p53 expression with favourable (3) and others with poor (7) prognosis in squamous cell carcinoma, whilst others still show no correlation at all (8). The same contradictory results have been reported in studies on adenocarcinoma (9). One reason is that the active, mutant or inactive p53 tumor suppressor may have been measured. In cervical cancer, the HPV E6 and E7 proteins bind and eventually degrade p53. The cyclin-dependent kinase inhibitor p27 blocks the G1/S transition necessary for cell cycle progression, thereby acting as a tumor suppressor. In squamous cell carcinoma, low p27 expression has been associated with poor prognosis (7, 10) or has been of no prognostic value (3). Similar results have been found in adenocarcinoma (9) and the role of p27, if any, is at present unclear. Loss of p27 expression has, however, also been correlated with late-stage cancer which will confound the results of prognostic studies unless adjusted for. COX-2 is the key regulatory enzyme in the conversion of arachidonic acid to prostaglandins. COX-2 has been associated with a variety of mechanisms in carcinogenesis, such as decreased apoptosis, inflammatory response to tumors, tumor invasion and neoangiogenesis. In squamous cell cancer, a high expression seems to correlate with poor prognosis (3, 11). Similar results have been found in adenocarcinoma, but might be confounded by higher expression in late-stage adenomatous cervical cancer (12). The results of the present study cannot determine if there are clinical implications. Differences in expression of tumor markers in tumors from pre- and postmenopausal women might indicate that there could be differences in carcinogenesis and maybe in their roles as prognostic predictors. Larger studies are warranted to address these questions, not the least as an increasing number of pharmaceuticals directed against tumor proteins are manufactured. It must be clarified whether these could have different effects in different age groups.

References

1 Gustafsson L, Ponten J, Bergström R and Adami H-O: International incidence rates of invasive cervical cancer before cytological screening. Int J Cancer *71*: 159-165, 1997.

2 Benedet JL, Bender H, Jones H 3rd, Ngan HY and Pecorelli S: FIGO staging classifications and clinical practice guidelines in the management of gynaecological cancers. FIGO Committee on Gynecologic Oncology. Int J Gynaecol Obstet *70*: 209-262, 2000.

3 Lindström AK, Stendahl U, Tot T, Lidström BM and Hellberg D: Predicting the outcome of squamous cell carcinoma of the uterine cervix using combinations of individual tumor marker expression. Anticancer Res *27*: 1609-1616, 2007.

4 Myers ER, McCrory DC, Nanada K, Bastian L and Matchar DB: Mathematical model for the natural history of human papillomavirus infection and cervical carcinogenesis. Am J Epidemiol *151*: 1158-1171, 2000.

5 Graflund M, Sorbe B, Sigurdardottir S and Karlsson M: HPV-DNA, vascular space invasion, and their impact on the clinical outcome in early-stage cervical carcinomas. Int J Gynecol Cancer *14*: 896-902, 2004.

6 Andersson S, Larson B, Hjerpe A, Silfverswärd C, Sällström J, Wilander E and Rylander E: Adenocarcinoma of the uterine cervix: the presence of human papillomavirus and the method of detection. Acta Obstet Gynecol Scand *82*: 960-965, 2003.

7 Oka K, Suzuki Y and Nakano T: Expression of p27 and p53 in cervical squamous cell carcinoma patients treated with radiotherapy alone. Cancer *88*: 2766-2773, 2000.

8 Shiohara S, Shiozawa T, Miyamoto T, Feng Y-Z, Kashima H, Kurai M, Suzuki A and Konishi I: Expression of cyclins, p53 and Ki-67 in cervical squamous cell carcinomas: overexpression of cyclin A is a poor prognostic factor in stage Ib and II disease. Virchows Arch *446*: 626-633, 2005.

9 Suzuki Y, Nakano T, Kato S, Ohno T, Tsujii H and Oka K: Immunohistochemical study of cell cycle-associated proteins in adenocarcinoma of the uterine cervix treated with radiotherapy alone: p53 status has a strong impact on prognosis. Int J Rad Oncol Biol Phys *60*: 231-236, 2004.

10 Kim JY, Lim SJ, Kim HJ, Shin E, Park K and Lee CM: Clinical significance of p27 and Skp2 protein expression in uterine cervical neoplasm. Int J Gynecol Pathol *26*: 242-247, 2007.

11 Ferrandina G, Lauriola L, Zannoni GF, Distefano MG, Legge F, Salutari V, Gessi M, Maggiano N, Scambia G and Raneletti FO: Expression of cyclooxygenase-2 (COX-2) in tumour and stroma compartments in cervical cancer: clinical implications. Br J Cancer *87*: 1145-1152, 2002.

12 Chen TH, Fukuhara K, Mandai M, Matsumura N and Kariya M: Increased cyclooxygenase-2 expression is correlated with suppressed antitumor immunity in cervical adenocarcinomas. Int J Gynecol Cancer *16*: 772-779, 2006.

Received January 23, 2008
Revised March 18, 2008
Accepted March 24, 2008