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ARTICLE

# Prognostic Significance of Dysadherin Expression in Cervical Squamous Cell Carcinoma

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**The protein and mRNA expression of dysadherin was studied in a series of squamous cell cervical carcinomas, and their clinicopathological associations and prognostic value were explored. Immunohistochemistry was used to assess protein expression of dysadherin in 206 patients with squamous cell cervical carcinoma, FIGO stage Ia-IVb. Frozen tissues from 20 cases in which the tumors showed variable dysadherin protein expression were used for laser capture microdissection (LCM) and processed for RT-PCR detection of dysadherin mRNA. Immunohistochemically, all the dysadherin-positive staining was membranous. Positive cell membranous dysadherin-positive staining was often observed at the edge of tumor nests, although strong immunoreactivity throughout whole tumor nests was also seen in some tumors. Basal cells of the normal cervical epithelia were positive for dysadherin while its expression in the squamous cell cervical carcinomas was variable. Among the 206 tumors, 23 (11.2%) were negative, 53 (25.7%) were scored 1+, 54 (26.2%) were scored 2+ and 76 (36.9%) were scored 3+. In the 20 tumors analyzed, mRNA expression of dysadherin basically corresponded to its protein expression. No significant correlation between expression of dysadherin and age, FIGO stage or lymph node status was observed. Higher level of dysadherin expression, however, was significantly associated with shorter overall survival (p=0.04). We conclude that there is dysadherin protein expression in basal and parabasal cells of normal cervical epithelia, and higher level of dysadherin protein expression in squamous cell cervical carcinoma is predictive of a shorter overall survival, indicating that dysadherin may be a valuable prognostic marker in cervical carcinoma.** (Pathology Oncology Research Vol 10, No 4, 212–218)

*Keywords:* dysadherin; squamous cell cervical carcinoma; immunohistochemistry; RT-PCR; laser capture microdissection

## *Introduction*

Cervical carcinoma is the second most frequent cause of cancer-related death in women.[1] At present, clinical stage and tumor volume are two crucial prognostic factors influencing the choice of treatment for cervical cancer patients.[2,3] However, cervical carcinomas with similar clinical stage and tumor size may have different aggressiveness and clinical courses, indicating a complex molecular mechanism for cervical cancer development. Therefore, it is necessary to search for better prognostic markers to improve clinical management of cervical carcinoma patients.

Abnormality in cell-cell adhesion is one of the basic features of carcinogenesis.[4] It has been verified that E-cadherin is involved in tumor progression.[5] Downregulation of E-cadherin expression results in reduction of cell-to-cell adhesiveness and promotes detachment of tumor cells from the primary lesion.[6] The molecular mechanism of inactivation of E-cadherin in human tumors is not fully understood. Many factors are involved in this process, such as DNA hypermethylation,[7,8] mutation of E-cadherin itself [9] and tyrosine phosphorylation of ß-catenin.[10]

Recently, a newly identified mechanism for inactivation of E-cadherin has been proposed. Dysadherin, an anti-

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adhesion molecule (a novel cancer-associated cell membrane glycoprotein) was characterized and denominated originally by Hirohashi et al.[11] It encodes 178 amino acids, including a putative signal sequence, an O-glycosylated extracellular domain, a single transmembrane domain and a short cytoplasmic tail. Transfection of a liver cancer cell line with the cDNA of dysadherin resulted in reduced cell-cell adhesiveness. At the same time, E-cadherin was greatly reduced in the transfected cells. After injection into mouse spleen, dysadherin transfectants formed a markedly larger number of intrahepatic metastatic nodules compared with the mock transfectants, suggesting a capacity of dysadherin to promote metastasis.[11]

At present, reports on the function and expression of dysadherin in tumors are still limited. It has been reported that dysadherin is highly expressed in colorectal cancers, and increased expression is significantly associated with lung metastasis and a poorer survival.[12] Similar results have been reported in pancreatic ductal adenocarcinoma[13] and thyroid carcinoma.[14] All these results pinpoint that dysadherin protein expression may become a new biological prognostic factor in human tumors.

To our best knowledge, no studies on dysadherin expression have been reported in cervical carcinoma. In the present study, we sought to clarify dysadherin expression patterns in a series of 206 squamous cell cervical carcinomas by immunohistochemistry, and explore its clinicopathologic associations. In addition, mRNA expression of dysadherin was also examined using Laser Capture Microdissection (LCM) assisted RT-PCR in 20 tumors with different levels of dysadherin protein expression by immunohistochemistry. We found that dysadherin was expressed in the basal cells of normal cervical epithelia, and higher level of dysadherin protein expression in squamous cell cervical carcinoma was predictive of a shorter overall survival. In addition, dysadherin mRNA and protein were expressed in parallel in the tumors.

## *Materials and Methods*

### *Patients and samples*

This study includes 206 patients with squamous cell cervical carcinoma, FIGO stage Ia-IVb. The patients were treated at Department of Gynecologic Oncology, The Norwegian Radium Hospital, during the period of 1995-1998. Staging was done according to the FIGO guidelines.[15] Patient and tumor parameters are listed in *Table 1*. The median age was 53.6 years, ranging 26-92 years.

Forty-nine patients with FIGO stage I tumor were treated with radical hysterectomy with pelvic lymphadenectomy. Of these, 17 had postoperative adjuvant treatment with radiation (7 patients), radiation and chemotherapy (2 patients) or chemotherapy (8 patients). Radiation to a pelvic field combined with brachytherapy was given to 119 patients with FIGO stage IIa-IVa tumors. Radiation to an extended field combined with brachytherapy was given to 9 patients with FIGO stage IIb-IVa tumor and para-aortic lymph node metastasis. Seven patients with FIGO stage IVb tumor were treated with chemotherapy and radiation consisting of external radiation combined with brachytherapy. A total of 22 patients in reduced performance status received either palliative radiotherapy (14) or no treatment (8). After treatment, patients were followed either at The Radium Hospital or at local hospitals. A total of 98

***Table 1.*** **Dysadherin expression in relation to clinicopathological parameters in cervical carcinoma**

| | *Total no.* | *Dysadherin protein expression (% in subgroup)* | | | | *P value* |
|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | |
| Age | | | | | | |
| ≤30 | 5 | 0 (0.0) | 1 (20.0) | 1 (20.0) | 3 (60.0) | |
| 31-50 | 83 | 7 (8.4) | 19 (22.9) | 20 (24.1) | 37 (44.6) | |
| 51-70 | 67 | 11 (16.4) | 15 (22.4) | 21 (31.3) | 20 (29.9) | |
| >70 | 51 | 5 ( 9.8) | 18 (35.3) | 12 (23.5) | 16 (31.4) | 0.45 |
| FIGO stage | | | | | | |
| I (Ia+Ib) | 61 | 7 (11.5) | 12 (19.7) | 14 (22.9) | 28 (45.9) | |
| II (IIa+II b) | 84 | 9 (10.7) | 23 (27.4) | 22 (26.2) | 30 (35.7) | |
| III (IIIa+IIIb) | 39 | 5 (12.8) | 11 (28.2) | 10 (25.6) | 13 (33.3) | |
| IV (IVa+ IVb) | 22 | 2 (9.1) | 7 (31.8) | 8 (36.4) | 5 (22.7) | 0.79 |
| Lymph node metastasis no. | | | | | | |
| 0 | 36 | 3 (8.3) | 8 (22.2) | 7 (19.4) | 18 (50.0) | |
| 1 | 6 | 0 (0.0) | 1 (16.7) | 2 (33.3) | 3 (50.0) | |
| 2 | 4 | 0 (0.0) | 1 (25.0) | 1 (25.0) | 2 (50.0) | |
| 3 | 3 | 0 (0.0) | 1 (33.3) | 1 (33.3) | 1 (33.3) | 0.99 |

*Table 2.* **Primers used for RT-PCR**

| *Primers* | *Genebank access no.* | *Primer location and sequences (bp)* | *Exon region* | *Product size (bp)* | *Annealing temperature (C)* |
|---|---|---|---|---|---|
| Dysadherin | AB072911 | F:134-154<br>5′ ACGTTGAAAGATACCACGTCC 3′(21)<br>R: 313-295<br>5′ ATCCGTTCCTTCCAGTTGC 3′(19) | 3~5 | 180 | 60 |
| GAPD | NM_002046 | F: 466~483<br>5′ TTCGTCATGGGTGTGAAC 3′(18)<br>R: 762~744<br>5′AGTGAGCTTCCCGTTCAGC 3′(19) | 5~7 | 297 | 60 |

(47.6%) patients died during the follow-up period. One patient was lost to follow-up after 3 years, and all others were followed until December 2003 with a median of 73 months (range 60 to 115 months).

During the pre-treatment evaluation, biopsies were taken. One half of them were snap frozen in liquid nitrogen and stored at -80ºC until use for RNA extraction, the other half was fixed in formalin and embedded in paraffin blocks for morphological and immunohistochemical examination. As normal controls we used sections from formalin-fixed, paraffin-embedded tissues from normal cervices from 10 patients (age range, 41-47) who underwent hysterectomy for benign reasons. All paraffin blocks were retrieved from the files of Department of Pathology, The Norwegian Radium Hospital. The histopathological diagnosis of specimens were re-evaluated by two experienced pathologists.

*Immunohistochemistry (IHC)*

Sections for immunohistochemistry were stained using the Dako EnVision™+ System, Peroxidase (DAB) (K4011, DakoCytomation, CA, USA) and Dakoautostainer. Deparaffinized sections were microwaved in 10 mM citrate buffer, pH 6.0, to unmask the epitopes. Sections were treated with 0.03% hydrogen peroxide for 5 minutes to block endogenous peroxidase. The sections were incubated with anti-dysadherin antibody (NCC-M53, 1:10,000 dilution) for 24 hours at 4°C. NCC-M53 was provided by Dr. Hirohashi (National Cancer Center Research Institute, Tokyo, Japan), and it has been characterized earlier.[13,16] The sections were then incubated for 30 minutes with peroxidase labeled polymer conjugated to goat anti-rabbit IgG. Tissues were stained for 5 minutes with 3'3-diaminobenzidine tetrahydrochloride (DAB), and then counterstained with hematoxylin, dehydrated, and mounted in Diatex. Paraffin-embedded human breast cancer cell line SK-BR-3 was used as positive control. Positively stained endothelial cells and lymphocytes in the same slide were used as internal positive controls. Negative controls included substitution of dysadherin antibody with normal mouse IgG at the same concentration as the antibody. All controls gave satisfactory results.

*Evaluation of IHC*

The samples were analyzed by 3 independent readers who were experienced in evaluating immunohistochemistry and had no knowledge of clinicopathological data of the patients. For each sample, usually more than 1000 cancer cells were analyzed. Immunoreactivity was evaluated according to the percentage of positively stained tumor cells relative to total tumor cells. The proportion of dysadherin-positive tumor cells was evaluated semiquantitatively and scored as follows: tumors with no positive or <5% positive tumor cells were scored as negative; tumors with 5-20% positive tumor cells were scored as 1+; tumors with 21-75% positive tumor cells were scored as 2+, and those with 76% and more positive tumor cells were scored as 3+.

*Laser capture microdissection*

Frozen tissues from 20 tumors immunohistochemically proved to have different levels of dysadherin protein expressions were selected for LCM. Briefly, 8 μm sections were fixed with acetone for 5 minutes, stained with hematoxylin-eosin (HE), dehydrated in graded alcohol and xylene, and then air-dried for 5 minutes. Small tumor areas (about 50 cells) on the section were selected and captured by using a PixCell laser capture microscope (Arcturus Engineering Inc., Mountain View, CA, USA). The LCM parameters were as follows: laser power of 70 milliwatts, laser pulse duration of 1.2-3.5 milliseconds and laser spot size of 7.5-15 μm diameter. The tissue section was overlaid with a thermoplastic polymer membrane mounted on optically transparent cap (CapSure, Arcturus Engineering Inc.), then the infrared laser was pulsed over the selected cells, and the cells were captured by focal melting of the membrane through laser activation.

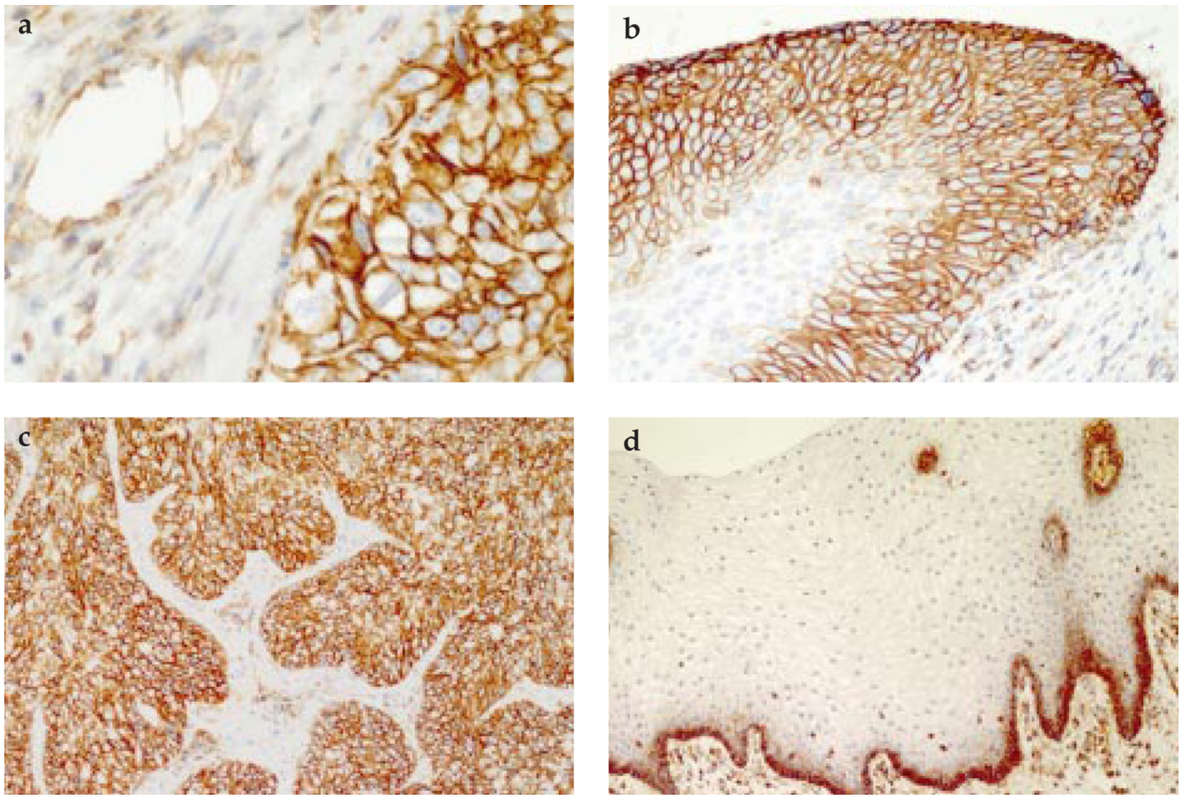


***Figure 1.*** *Immunohistochemical expression of dysadherin in cervical carcinomas.* ***(a):*** *Dysadherin immunostaining was rather heterogeneous, and was localized at the membranes of the majority of cancer cells as well as a portion of lymphocytes and endothelial cells (x40).* ***(b):*** *Positive staining localized at the periphery of a tumor nest (x20).* ***(c):*** *Positive staining distributed throughout the tumor nests (x20);* ***(d):*** *Dysadherin-positive staining localized at basal cells of stratified normal squamous epithelium (x20).*

*RNA Extraction*

The cells captured on the cap were microcentrifuged into an Eppendorf tube. Total RNA was then extracted by using an Absolutely RNA™ Nanoprep Kit (Stratagene Inc., La Jolla, CA, USA) according to the recommendations of the manufacturer.

*RT-PCR*

The Qiagen OneStep RT-PCR kit (Qiagen Inc., Valencia, CA, USA) was used for RT-PCR, which was performed in a 25 µl volume using 5 µl 5× Qiagen OneStep RT-PCR buffer, 5 µl 5× Q solution, 200 µM dNTP, 0.6 µM 3' primer, 0.6 µM 5' primer, 0.2 µM 3' GAPD primer, 0.2 µM 5' GAPD primer, 2 µl RNA, and 1 µl Qiagen OneStep RT-PCR enzyme mix. The reactions were carried out under the following conditions: 50°C for 30 min for reverse transcription of RNA, followed by 95°C for 15 minutes for cDNA denaturation, and then 40 cycles of 94°C for 60 sec, 60°C for 30 sec, and 72°C for 1 min. Final extension was performed at 72°C for 7 min. The features of all primers are given in *Table 2*. All primers spanned at least one intron. GAPD primers were used in each reaction as internal control. Breast cancer cell line (MDA-MB-231) was used as positive control, and negative controls were performed using water instead of primer. The intensity of the amplified band of dysadherin in each sample was analyzed and compared with that of GAPD PCR band, which was added into the same RT-PCR reaction system. The ratio of the intensities of dysadherin and GAPD PCR bands was semiquantitatively scored as high or low.

*Statistical analysis*

The association between the expressed proteins and between protein expression and clinicopathological parameters was tested by the $\chi^2$ test. Overall survival was calculated from date of diagnosis to date of death or December 1, 2003, using the method of Kaplan-Meier. The log-rank test with a test for trend in the case of ordered variables was used for univariate, and a Cox proportional haz-

ards regression model for multivariate analysis of survival. A backward stepwise procedure was used. The hazard proportionality was verified by computing the log minus log against time. The SPSS statistical package (SPSS Inc, Chicago, IL, USA) was used for the statistical analysis. P values of <0.05 were considered statistically significant.

## *Results*

### *Protein expression of dysadherin*

The majority of the tumors were positive for dysadherin immunostaining. In the positive tumors, membranous dysadherin immunostaining was rather heterogeneous (*Figure 1a*). In addition to tumor cells, a portion of normal cells such as lymphocytes, Langerhans and endothelial cells were also dysadherin-positive. Positive dysadherin immunostaining was usually localized at the periphery of the tumor nests (*Figure 1b*), although extensive staining throughout the whole tumor nests was also seen in some tumors (*Figure 1c*). Positive membranous dysadherin immunostaining was observed on all the basal cells of the 10 normal cervical epithelia (*Figure 1d*). There was variable dysadherin protein expression observed in the squamous cell cervical carcinomas. Among the 206 tumors, 23 (11.2%) were negative, 53 (25.7%) were scored 1+, 54 (26.2%) 2+ and 76 (36.9%) 3+.

### *Correlation to clinicopathological parameters*

Dysadherin protein expression was not associated with either age or FIGO stage. In patients who underwent surgery, no correlation was seen between the expression of dysadherin protein and lymph node metastasis. The relationships between the clinicopathological characteristics of patients and protein expressions are summarized in *Table 1*.

### *Dysadherin mRNA expression*

The 20 selected tumors were successfully examined using the LCM-assisted RT-PCR method (*Figure 2*). Among these tumors, 10 were immunohistochemically scored as 3+ for dysadherin, 3 as 2+, 2 as 1+, and 5 were negative. Tumors with 3+ immunostaining for dysadherin demonstrated higher intensity of dysadherin RT-PCR bands than those with 1+ or 2+. There was no dysadherin RT-PCR band in the tumors with dysadherin-negative immunostaining (*Figure 3*), indicating that mRNA expression basically corresponded to protein expression.

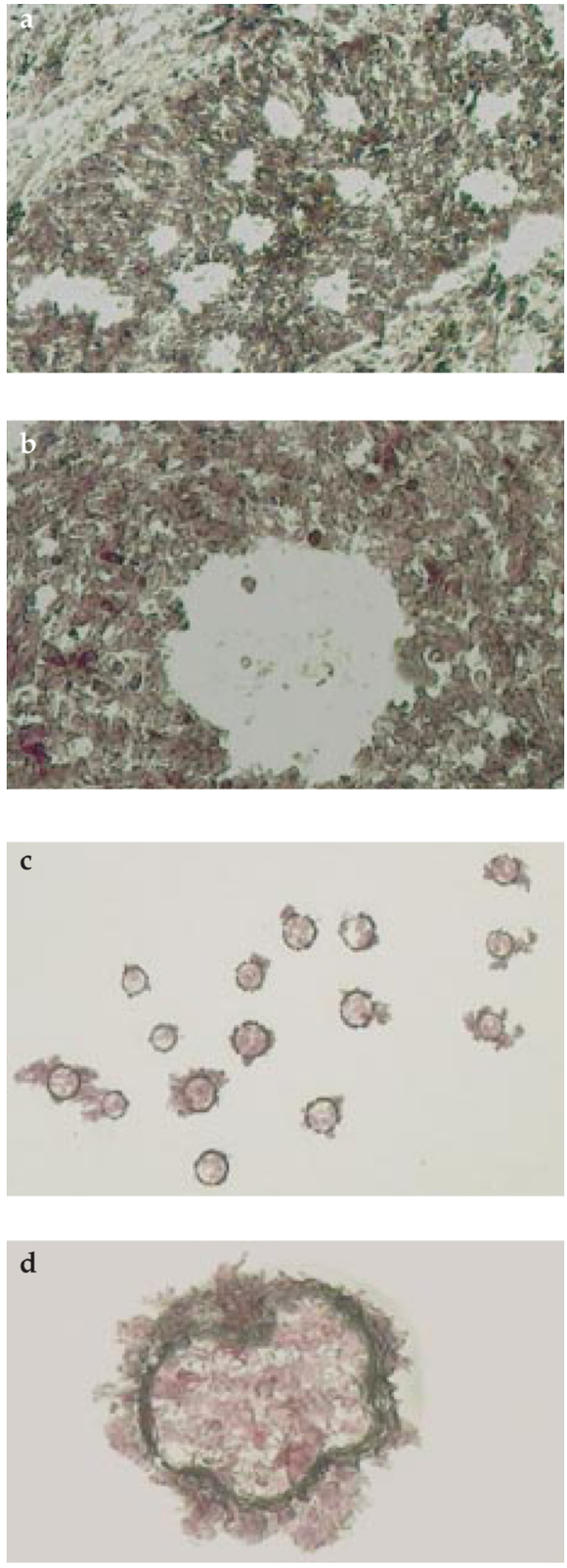


***Figure 2.*** *LCM of cervical carcinoma cells from HE-stained sections (8 m) (x20). Photographs of the surrounding tissues that remain on slides after LCM* ***(a,b)*** *and about 50 captured cancer cells on cap* ***(c,d)****.*

*Survival analyses*

Kaplan-Meier estimate of survival association showed that patients with tumors immunohistochemically scored as 2+ or lower dysadherin protein expression had a significantly longer overall survival than those with their tumor scored as 3+ expression (p=0.04) (*Figure 4*).

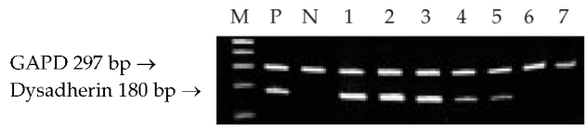


***Figure 3.*** *RT-PCR analysis of captured cancer cells for dysadherin. (M): 100 bp ladder marker; (P): positive control (MDA-MB-231 cell line); (N): negative control (using water instead of primer). The size of RT-PCR product is indicated by arrow: dysadherin (180 bp), GAPD (297 bp). Lanes 1-3: samples with grade 3 immunostaining of dysadherin showing high level of EphA2 mRNA expression. Lanes 4,5: samples with grade 2 or 1 immunostaining of dysadherin showing moderate level of EphA2 mRNA expression. Lanes 6,7: samples with grade 0 immunostaining of dysadherin showing no EphA2 mRNA expression.*

## *Discussion*

The cadherin cell-cell adhesion system is well known to be involved in the maintenance of normal cell-cell adhesion. The most studied member of the cadherin family is E-cadherin, a glycoprotein that binds epithelial cells to each other by calcium-dependent interactions.[17] It has been reported that E-cadherin is expressed on cellular membrane of the basal and parabasal cells of normal cervical squamous epithelium,[18] which is in line with our present finding of dysadherin protein expression. Immunohistochemically, we have shown in the present study that membranous dysadherin protein expression is confined to the basal and parabasal cells of the normal cervical epithelia, supporting the notion that dysadherin is a transmembrane protein similarly to E-cadherin.

Dysadherin expression has been shown to be tumor-associated in many cancer tissues.[11, 13] In our present study, dysadherin expression was found in the majority of squamous cell cervical carcinomas (88.8%), and was generally localized at the cell membrane as detected by immunohistochemistry. In some well-differentiated tumors, tumor cells at the periphery of tumor nests were positive for dysadherin protein expression, while in some other, poorly differentiated ones tumor cells throughout whole tumor nests were positive for dysadherin, indicating its association with histological grade. Similar results have been reported by others, in which dysadherin was more frequently overexpressed in dissociating or infiltrative tumor cells than in well-differentiated tumor nests in colorectal carcinoma[11] and pancreatic ductal adenocarcinoma.[13] Statistical analysis, however, showed no association between dysadherin protein expression and histological grades.

It has been reported that the loss of E-cadherin function in cervical carcinoma results in higher capability for tumor invasion and progression.[18] Dysadherin has been shown to be able to inactivate and downregulate E-cadherin at post-transcriptional level in liver cancer cells, and promote aggressiveness and metastasis.[11] Thus, it is likely that dysadherin function is one of the mechanisms underlying the dysfunction of cell-cell adhesion system in cervical carcinoma as well. At present, the regulating mechanisms of cell-cell adhesion with regard to E-cadherin and dysadherin communication[4] in cervical carcinoma are far from being understood, and further studies are needed to clarify the molecular role of dysadherin in cervical cancer initiation and development.

It has been reported that dysadherin expression is associated with tumor aggressiveness, metastasis and prognosis of patients in some tumors types, such as advanced colorectal carcinoma,[12] pancreatic ductal adenocarcinoma[13] and thyroid carcinoma.[14] In our present study, no significant association between dysadherin protein expression and age, FIGO stage or lymph node metastasis was observed. However, we found that higher level of dysadherin protein expression was significantly associated with a shorter overall survival (p=0.04). To date, this is the first report on dysadherin expression and its clinicopathological association in cervical carcinoma.

To determine whether the mRNA level of dysadherin corresponded to its protein expression, we collected about 50 tumor cells each from 20 tumors by laser capture

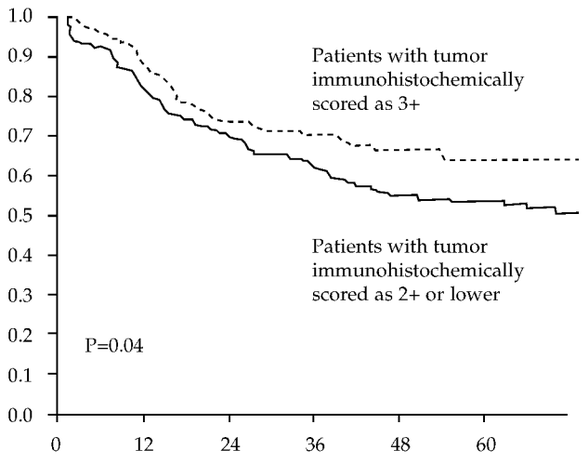


***Figure 4.*** *Kaplan-Meier analysis of overall survival related to dysadherin expression. Patients with tumors expressing lower levels (≤2+) dysadherin protein showed a significantly better survival curve than those with tumors expressing 3+ dysadherin protein (p=0.04).*

microdissection (LCM), and the cells were used for RNA extraction and gene-specific RT-PCR. Our results show that mRNA level of dysadherin basically corresponds to its protein expression, indicating that post-transcriptional regulation of dysadherin expression plays little role in cervical carcinoma.

We conclude that dysadherin protein expression is confined to basal and parabasal cells in normal cervical epithelia, parallel dysadherin mRNA and protein expressions exist in squamous cell cervical carcinoma, and high level of dysadherin protein expression is predictive of a shorter overall survival, indicating that dysadherin may be a valuable prognostic marker for cervical carcinoma.

### *Acknowledgement*

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### *References*

1. *Jemal A, Murray T, Samuels A et al:* Cancer statistics, 2003. CA Cancer J Clin 53:5-26, 2003
2. *Flores-Luna L, Salazar-Martinez E, Escudero-De los Rios P et al:* Prognostic factors related to cervical cancer survival in Mexican women. Int J Gynaecol Obstet 75:33-42, 2001
3. *Kawagoe T, Kashimura M, Matsuura Y et al:* Clinical significance of tumor size in stage IB and II carcinoma of the uterine cervix. Int J Gynecol Cancer 9:421-426, 1999
4. *Hirohashi S, Kanai Y:* Cell adhesion system and human cancer morphogenesis. Cancer Sci 94:575-581, 2003
5. *Birchmeier W.* E-cadherin as a tumor (invasion) suppressor gene. Bioessays 17:97-99, 1995
6. *Christofori G, Semb H:* The role of the cell-adhesion molecule E-cadherin as a tumor-suppressor gene. Trends Biochem Sci 24:73-76, 1999
7. *Yoshiura K, Kanai Y, Ochiai A et al:* Silencing of the E-cadherin invasion-suppressor gene by CpG methylation in human carcinomas. Proc Natl Acad Sci USA 92:7416-7419, 1995
8. *Graff JR, Gabrielson E, Fujii H et al:* Methylation patterns of the E-cadherin 5' CpG island are unstable and reflect the dynamic, heterogeneous loss of E-cadherin expression during metastatic progression. J Biol Chem 275:2727-2732, 2000
9. *Becker KF, Atkinson MJ, Reich U et al:* E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. Cancer Res 54:3845-3852, 1994
10. *Matsuyoshi N, Hamaguchi M, Taniguchi S et al:* Cadherin-mediated cell-cell adhesion is perturbed by v-src tyrosine phosphorylation in metastatic fibroblasts. J Cell Biol 118:703-714, 1992
11. *Ino Y, Gotoh M, Sakamoto M et al:* Dysadherin, a cancer-associated cell membrane glycoprotein, down-regulates E-cadherin and promotes metastasis. Proc Natl Acad Sci USA 99:365-370, 2002
12. *Aoki S, Shimamura T, Shibata T et al:* Prognostic significance of dysadherin expression in advanced colorectal carcinoma. Br J Cancer 88:726-732, 2003
13. *Shimamura T, Sakamoto M, Ino Y et al:* Dysadherin overexpression in pancreatic ductal adenocarcinoma reflects tumor aggressiveness: relationship to e-cadherin expression. J Clin Oncol 21:659-667, 2003
14. *Sato H, Ino Y, Miura A et al:* Dysadherin: expression and clinical significance in thyroid carcinoma. J Clin Endocrinol Metab 88:4407-4412, 2003
15. *Pecorelli S, Odicino F:* Cervical cancer staging. Cancer J 9:390-394, 2003
16. *Tsuiji H, Takasaki S, Sakamoto M et al:* Aberrant O-glycosylation inhibits stable expression of dysadherin, a carcinoma-associated antigen, and facilitates cell-cell adhesion. Glycobiology 13:521-527, 2003
17. *Adams CL, Nelson WJ, Smith SJ:* Quantitative analysis of cadherin-catenin-actin reorganization during development of cell-cell adhesion. J Cell Biol 135:1899-1911, 1996
18. *Carico E, Atlante M, Bucci B et al:* E-cadherin and alpha-catenin expression during tumor progression of cervical carcinoma. Gynecol Oncol 80:156-161, 2001