DNA 损伤修复相关基因 *Polζ*、*ERCC1*、*ERCC2* 和 *RAD52* 在宫颈癌中的表达及其临床意义

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摘　要:[目的] 评价 DNA 损伤修复相关基因在宫颈癌组织中的表达及其临床意义。[方法] 纳入 2008~2009 年在复旦大学附属肿瘤医院行根治性手术并辅助同步放化疗的 113 例宫颈鳞状细胞癌患者，采用免疫组化法检测 DNA 损伤修复相关基因（*Polζ*,*ERCC1*,*ERCC2* 和 *RAD52*）在石蜡包埋组织中的蛋白表达水平。[结果] Polζ,ERCC1,ERCC2 和 RAD52 的阳性表达率分别为 22.1%,46.0%,48.7%和 20.4%。Kaplan-Meier 生存分析表明 Polζ 蛋白表达阳性的患者无进展生存期更短(32 个月 *vs* 34 个月,*P*=0.008)。多因素生存分析显示 Polζ 是肿瘤复发的重要因素(adjusted HR=7.79,95%CI:2.21~27.52,*P*=0.001)。[结论] Polζ 可作为宫颈癌判断预后的预测因素,这可能是由于宫颈癌患者潜在的放化疗抵抗导致的,该机制值得进一步研究。

关键词:宫颈癌;预后;Polζ;ERCC1;ERCC2;RAD52
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The Clinical Significance of Polζ,ERCC1,ERCC2 and RAD52 Expression in Cervical Cancer Tissues

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Abstract:[Purpose] To investigate the expression profiles of multiple DNA damage repair genes Polζ,ERCC1,ERCC2 and RAD52 in cervical cancer tissues and its clinical significance. [Methods] A total of 113 patients with squamous cell carcinoma of cervical cancer,who had adjuvant concurrent chemoradiation therapy after radical surgery treated at Fudan University Shanghai Cancer Center between 2008 and 2009 were enrolled,and immunohistochemistry was used to test Polζ,ERCC1,ERCC2 and RAD52 protein expression in paraffin-embedded tissues. [Results] The positive expression of Polζ,ERCC1,ERCC2 and RAD52 was 22.1%,46.0%,48.7% and 20.4%, respectively. Kaplan-meier survival showed that the patients with Polζ-positive expression had a significantly shorter progression-free survival(32 months *vs* 34 months,*P*=0.008). Multivariate Cox proportional hazards regression analysis revealed that Polζ protein expression (adjusted HR = 7.79,95% CI:2.21~27.52,*P*=0.001) was a significant predictor for recurrence/metastasis. [Conclusions] Polζ expression can serve as the predictor for poor prognosis in cervical cancer,which might due to the chemoradiation resistance of cervical cancer patients. The exact mechanism needs further research.

Key words:cervical cancer;prognosis;Polζ;ERCC1;ERCC2;RAD52

宫颈癌是发展中国家发病率最高的女性生殖道恶性肿瘤,占全球女性肿瘤发病率的第 5 位,死亡率的第 4 位[1]。目前普遍认为宫颈癌的发生是多种因素的综合作用,如遗传学因素、生物学因素和 HPV 病毒感染等[2]。这些因素造成了抑癌基因的突变或缺失、癌基因的异常扩增和表达。机体通过 DNA 损伤修复基因的多种修复途径参与损伤的修复并保持基因组的稳定性，而这些基因也会造成机体对放化疗等的抵抗。DNA 损伤修复等放化疗反应相关基因

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的表达可能作为肿瘤的预后指标并指导临床治疗方案的选择。为探讨相关基因表达与宫颈癌预后之间的关系，我们检测了 DNA 损伤修复相关蛋白 DNA 聚合酶 ζ(DNA polymerase ζ,Polζ),切除修复交叉互补基因 1(excision repair cross complementing 1,ERCC1),切除修复交叉互补基因(excision repair cross complementing 2,ERCC2) 和 RAD52 在宫颈癌组织中的表达水平。

1 资料与方法

1.1 研究对象

收集 2008~2009 年在复旦大学附属肿瘤医院宫颈鳞状细胞癌 (squamous cell carcinoma of cervicalcancer,CSCC)中 FIGO(International Federation of Gynecology and Obstetrics) 分期ⅠB-ⅡB 期的患者 113 例，所有患者均行根治性子宫切除及盆腔淋巴结清扫术，术后病理均提示高危因素并行辅助性同步放化疗。组织标本用 4%的甲醛溶液固定,常规脱水,石蜡包埋。

1.2 组织芯片制作

对每个蜡块进行切片和 HE 染色，标记典型的肿瘤及正常组织。由复旦大学附属肿瘤医院组织库制作 10×12 排列组织微阵列，以 5μm 厚度连续切片。每点组织结构完整、排列整齐,无脱片、变形、缺损现象。

1.3 免疫组织化学染色

所有抗体均购于美国 Santa Cruz 公司，包括 Polζ、ERCC1、ERCC2 和 RAD52 等 4 种抗体。免疫组织化学试剂盒购自丹麦 DAKO 公司,按照试剂盒说明书操作。以已知阳性切片作为阳性对照，以正常非免疫的鼠/兔血清代替第一抗体作为阴性对照。

1.4 结果判断

由两名经验丰富的妇科病理医师采用盲法在显微镜下观察免疫组化染色结果。根据细胞染色强度及染色细胞百分率综合分析评定：根据染色强度分为 1+~3+，根据细胞核染色比例分为 1+(染色细胞数<10%),2+(染色细胞数 10%~50%),3+(染色细胞数≥50%)。根据染色强度及染色细胞百分率综合评分为 0(<1+),1(1+~2+),2(>2+~4+),3(>4+~6+)。综合评分 2+以下为阴性,2+以上为阳性。

1.5 统计学处理

采用 SPSS18.0 软件进行数据分析。无进展生存期(progression-free survival,PFS)为手术时间至肿瘤复发时间。随访截止时间为 2011 年 7 月。在最后随访日期时未进展、失访或因其他原因死亡视为截尾。采用 Pearson's χ^2 检验分析蛋白表达强度与临床病理因素之间的关系。采用 Kaplan-Meier 生存分析及 Log-rank 检验绘制生存曲线并计算生存率。采用 Cox 比例风险模型分析预后因素与生存及复发之间的关系。P<0.05 为差异有统计学意义。

2 结 果

2.1 DNA 损伤修复基因在宫颈癌中的表达

ERCC1 及 ERCC2 表达阳性率较高,阳性率分别为 46.0%及 48.7%,Polζ 及 RAD52 表达阳性率相对较低,阳性率分别为 22.1%及 20.4%(Figure 1,Table 1)。

2.2 DNA 损伤修复基因与宫颈癌临床病理因素的关系

运用 χ^2 检验判断 4 种蛋白表达强度与 FIGO 分期、肿瘤大小、盆腔淋巴结、脉管浸润、肌层浸润深度及肿瘤复发之间的关系,结果显示 Polζ 表达阳性率与肿瘤大小、脉管浸润深度及肿瘤复发相关 (P<0.05),ERCC1 表达高低与 FIGO 分期相关(P<0.05),其他基因的表达与临床病理因素无关(Table 1)。

2.3 DNA 损伤修复基因与宫颈癌预后之间的关系

宫颈癌患者 113 例的 3 年无进展生存率为 27.4%,中位 PFS 为 33 个月。Kaplan-Meier 生存分析显示 Polζ 表达阳性患者中位 PFS 低于 Polζ 表达阴性患者 (32 个月 *vs* 34 个月,Log-rank 检验,P=0.008),ERCC1 表达阳性患者中位 PFS 与 ERCC1 表达阳性患者相当 (32 个月 *vs* 34 个月,P>0.05),ERCC2 表达阳性患者中位 PFS 与 ERCC2 表达阳性患者相当(33 个月 *vs* 34 个月,P>0.05),RAD52 表达阳性患者中位 PFS 与 RAD52 表达阳性患者相当 (33 个月 *vs* 34 个月,P>0.05)(Figure 2)。

采用 Cox 比例风险模型同时进行单因素及多因素分析评估宫颈癌的预后因素,结果发现 Polζ 表达阳性率是宫颈鳞状细胞癌复发的预后因素 (HR=7.79,95%CI:2.21~27.52,P=0.001)(Table 2)。

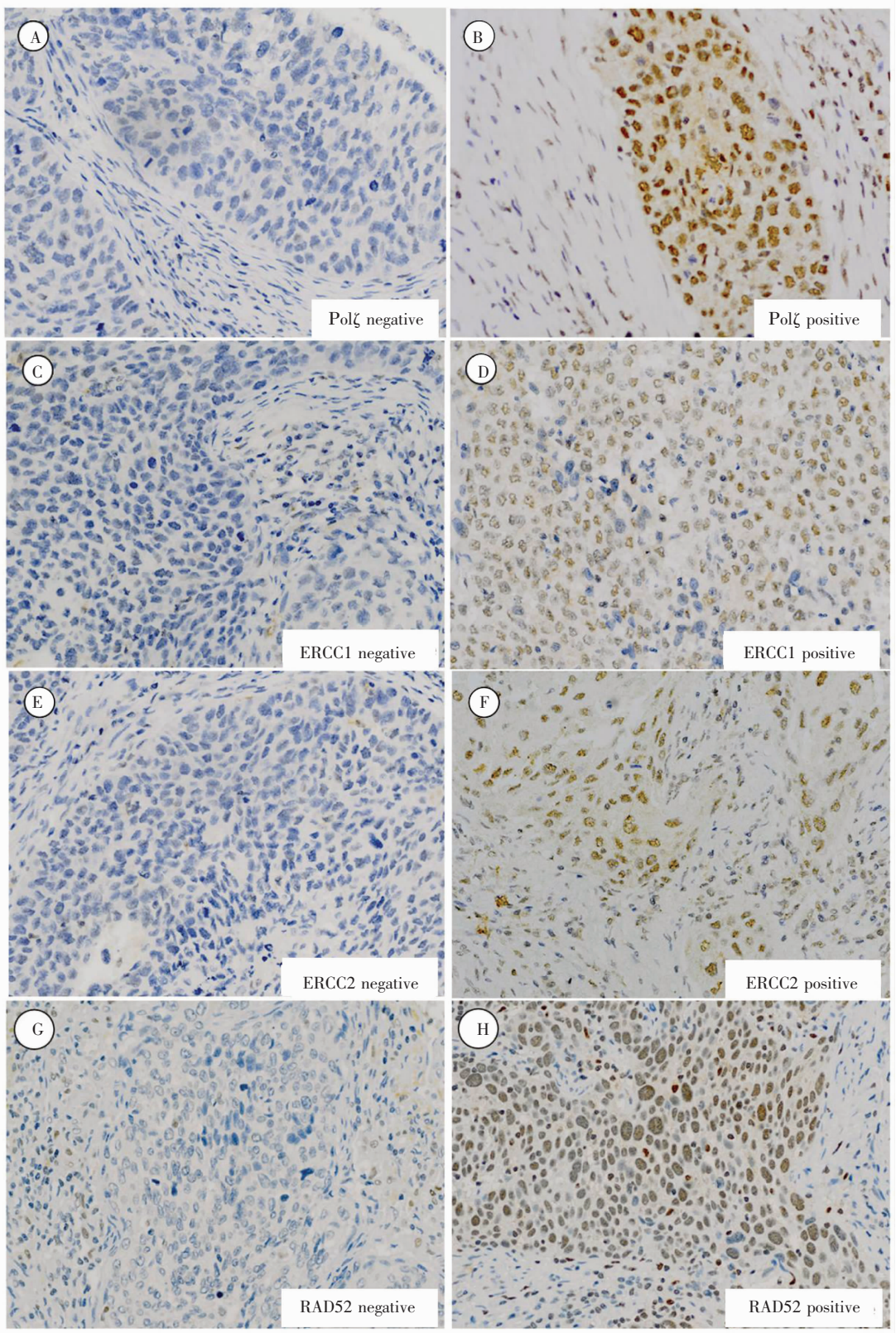

Figure 1　Expression of Polζ, ERCC1, ERCC2 and RAD52 proteins in CSCC tissues by immunohistochemistry assay

Table 1 The associations of clinicopathological characteristics with expression of Polζ, ERCC1, ERCC2 and RAD52 proteins in CSCC

Characteristics	N (%)	Pol ζ			ERCC1			ERCC2			RAD52		
		Negative(%)	Positive(%)	*P*	Negative(%)	Positive(%)	*P*	Negative(%)	Positive(%)	*P*	Negative(%)	Positive(%)	*P*
All patients	155	88 (77.9)	25 (22.1)		61 (54.0)	52 (46.0)		58 (51.3)	55 (48.7)		90 (79.7)	23 (20.4)	
FIGO stage				0.403			0.011			0.302			0.827
Ⅰ B	53 (47.3)	42 (79.3)	11 (20.8)		21 (39.6)	32 (60.4)		26 (49.1)	27 (50.9)		43 (81.1)	10 (18.9)	
Ⅱ A	52 (46.4)	41 (78.9)	11 (21.2)		35 (67.3)	17 (32.7)		30 (57.7)	22 (42.3)		41 (78.9)	11 (21.2)	
Ⅱ B	7 (6.3)	4 (57.1)	3 (42.9)		5 (71.4)	2 (28.6)		2 (28.6)	5 (71.4)		5 (71.4)	2 (28.6)	
Tumor size(cm)				0.049			0.815			0.835			0.295
≤ 4	40 (35.4)	27 (67.5)	13 (32.5)		21 (52.5)	19 (47.5)		20 (50.0)	20 (50.0)		34 (85.0)	6 (15.0)	
> 4	73 (64.6)	61 (83.6)	12 (16.4)		40 (54.8)	33 (45.2)		38 (52.1)	35 (48.0)		56 (76.7)	17 (23.3)	
Pelvic LN				0.984			0.153			0.134			0.688
Negative	68 (60.2)	53 (77.9)	15 (22.1)		33 (48.5)	35 (51.5)		31 (45.6)	37 (54.4)		55 (80.9)	13 (19.1)	
Positive	45 (39.8)	35 (77.8)	10 (22.2)		28 (62.2)	17 (37.8)		27 (60.0)	18 (40.0)		35 (77.8)	10 (22.2)	
LVSI				0.230			0.678			0.200			0.191
Negative	65 (57.5)	48 (73.9)	17 (26.2)		34 (52.3)	31 (47.7)		30 (46.2)	35 (53.9)		49 (75.4)	16 (24.6)	
Positive	48 (42.5)	40 (83.3)	8 (16.7)		27 (56.3)	21 (43.8)		28 (58.3)	20 (41.7)		41 (85.4)	7 (14.6)	
Depth of cervical stroma invasion				0.021			0.375			0.671			0.400
≤2/3	16 (14.2)	16 (100)	0 (0)		7 (43.8)	9 (56.3)		9 (56.3)	7 (43.8)		14 (87.5)	2 (12.5)	
> 2/3	97 (85.8)	72 (74.2)	25 (25.8)		54 (55.7)	43 (44.3)		49 (50.5)	48 (49.5)		76 (78.4)	21 (21.7)	
Recurrence				0.007			0.749			0.498			0.190
No	99 (87.6)	81 (81.8)	18 (18.2)		54 (54.6)	45 (45.5)		52 (52.5)	47 (47.5)		77 (77.8)	22 (22.2)	
Yes	14 (12.4)	7 (50.0)	7 (50.0)		7 (50.0)	7 (50.0)		6 (42.9)	8 (57.1)		13 (92.9)	1 (7.1)	

CSCC: squamous cell carcinoma of cervical cancer, Polζ: DNA polymerase ζ, ERCC1: excision repair cross complementing 1, ERCC2: excision repair cross complementing 2, FIGO International Federation of Gynecology and Obstetrics, LN: lymph node, LVSI: lympho-vascular space invasion.

3 讨 论

目前 DNA 损伤修复系统主要有错配修复 (mismatch repair, MMR)、碱基切除修复(base excision repair, BER)、核苷酸切除修复(nucleotide excision repair, NER)以及双链断裂修复 (double strand breaks repair, DSBR)等[3], 另一途径跨损伤 DNA 合成通路(translesion DNA synthesis, TLS)可在细胞的严重损伤修复适应中发挥作用, 属于一类复制后修复过程[4]。ERCC1 和 ERCC2 是 NER 的主要成员, ERCC1 参与 DNA 链的损伤识别和切割, ERCC2 发挥 DNA 解螺旋作用。DSBR 有两种主要修复途径：一是非同源末端连接(non-homologous end joining, NHEJ)；二是同源重组修复 (homologous recombination repair, HRR) [5]。在 HRR 的不同阶段, *RAD52* 基因起着非常重要的作用, 与同源互补序列的退火密切相关 [6]。Polζ 是 TLS 的主要参与者[7]。当 DNA 链在复制过程中遇到损伤 DNA 而使复制停顿时, 机体能通过 TLS 以忽略存在的损伤, 继续进行 DNA 复制, 使得一些损伤在基因组中暂时保留下来。这样一方面能维持细胞基因组的稳定, 提高细胞对 DNA 损伤剂杀细胞效应的抵抗性; 另一方面则允许损伤在基因组中存在, 易于产生突变, 使细胞在毒性刺激的选择压力下能够慢慢适应, 对 DNA 损伤产生一定程度的耐受[8,9]。

既往报道 ERCC1 表达与食管癌、胃癌、结肠癌、非小细胞肺癌等的预后相关 [10,11], ERCC2 单核苷酸多态性与肺癌、结直肠癌、乳腺癌等的预后相关 [10,12,13], 而

Table 2 The associations of Polζ, ERCC1, ERCC2 and RAD52 proteins expression with recurrence in CSCC

Prognostic factors	N (%)	Nonrecurrence N (%)	Recurrence N (%)	Univariate		Multivariate	
				HR (95% CI)[a]	P^a	HR (95% CI)[b]	P^b
All patients	113 (100)	99 (87.6)	14 (12.4)				
FIGO stage							
ⅠB	53 (47.3)	47 (88.7)	6 (11.3)	1.00		1.00	
ⅡA	52 (46.4)	47 (90.4)	5 (9.6)	0.87 (0.26~2.84)	0.812	0.93 (0.28~3.15)	0.912
ⅡB	7 (6.3)	4 (57.1)	3 (42.9)	4.95 (1.23~19.87)	0.024	3.17 (0.67~15.04)	0.146
Tumor size(cm)							
≤ 4	40 (35.4)	38 (95.0)	2 (5.0)	1.00		1.00	
> 4	73 (64.6)	61 (83.6)	12 (16.4)	3.53 (0.79~15.76)	0.099	4.19 (0.92~19.13)	0.065
Pelvic LN							
Negative	68 (60.2)	59 (86.8)	9 (13.2)	1.00		1.00	
Positive	45 (39.8)	40 (88.9)	5 (11.1)	0.84 (0.28~2.50)	0.753	0.56 (0.16~1.97)	0.362
LVSI							
Negative	65 (57.5)	58 (89.2)	7 (10.8)	1.00		1.00	
Positive	48 (42.5)	41 (85.4)	7 (14.6)	1.44 (0.50~4.09)	0.500	2.10 (0.63~6.98)	0.225
Depth ofcervical stromal invasion							
≤ 2/3	16 (14.2)	14 (87.5)	2 (12.5)	1.00		1.00	
> 2/3	97 (85.8)	85 (87.6)	12 (12.4)	1.00 (0.23~4.49)	0.996	0.92 (0.20~4.16)	0.909
Pol ζ expression status							
Negative	88 (77.9)	81 (92.1)	7 (8.0)	1.00		1.00	
Positive	25 (22.1)	18 (72.0)	7 (28.0)	3.76 (1.32~10.74)	0.013	7.79 (2.21~27.52)	0.001
ERCC1 expression status							
Negative	61 (54.0)	54 (88.5)	7 (11.5)	1.00		1.00	
Positive	52 (46.0)	45 (86.5)	7 (13.5)	1.22 (0.43~3.46)	0.716	1.74 (0.56~5.43)	0.342
ERCC2 expression status							
Negative	58 (51.3)	52 (89.7)	6 (10.3)	1.00		1.00	
Positive	55 (48.7)	47 (85.5)	8 (14.6)	1.46 (0.51~4.21)	0.484	1.61 (0.54~4.81)	0.397
RAD52 expression status							
Negative	90 (79.7)	77 (85.6)	13 (14.4)	1.00		1.00	
Positive	23 (20.4)	22 (95.7)	1 (4.4)	0.28 (0.04~2.17)	0.225	0.25 (0.03~1.96)	0.188

LN: lymphnode, LVSI: lympho-vascular space invasion, Cox proportional hazards regression analysis [a]: without adjustment and [b]: with adjustment for FIGO stage, tumor size, Pelvic LN, LVSI, and depth of stromal invasion

RAD52 表达与鼻咽癌预后相关[14]。本研究未发现 ERCC1、ERCC2 及 RAD52 表达与宫颈癌预后相关。

既往研究表明在结肠癌细胞、卵巢癌细胞、鼻咽癌细胞、肺癌细胞、人胶质瘤细胞中 REV3L 的高表达会导致铂类化疗药物疗效降低，并出现药物抵抗[8,9,15~18]。在本研究中，我们发现 Polζ 蛋白表达阳性率与肿瘤大小、脉管浸润深度及肿瘤复发相关。而且，Polζ 蛋白表达阳性组与阴性组相比 PFS 更短，这与既往研究相符。我们推测，Polζ 可能通过介导宫颈癌对放化疗的抵抗影响患者预后，其机制需进一步探讨。

总之，本研究显示 Polζ 与宫颈癌患者预后相关，Polζ 可能作为宫颈癌放化疗敏感性及预后的判断指标，并进一步可能作为宫颈癌治疗的分子靶点。这可能是由于宫颈癌患者潜在的放化疗抵抗导致的，其具体作用机制有待进一步研究。

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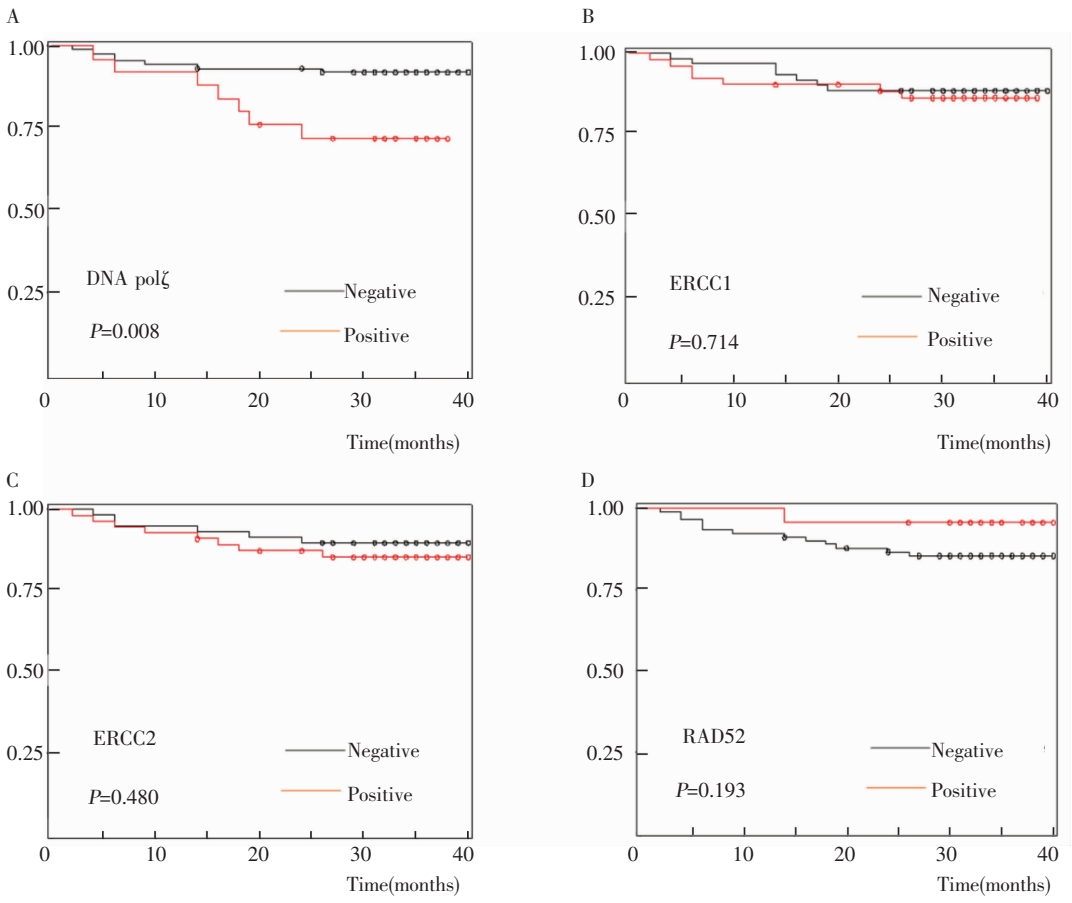

Figure 2　Kaplan-Meier survival analysis of progression-free survival in CSCCwith different status of Polζ (A),ERCC1 (B),ERCC2 (C),and RAD52 (D)expression

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