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Distribution of *CCND1* A870G Polymorphism in Patients with Advanced Uterine Cervical Carcinoma

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Abstract We examined the distribution of the *CCND1* A870G (rs9344) polymorphic variant in patients with cervical cancer (n=129) and healthy individuals (n=288) in a sample of a Polish cohort. We showed that patients with advanced cervical cancer bearing the *CCND1* A/A and A/G genotypes displayed a 1.811-fold increased risk of cervical cancer (95% CI=1.150–2.852, p=0.0098). We also found a significantly higher frequency of the *CCND1* 870A allele in patients with cancer than in controls, p=0.0116. Our investigation confirmed that the *CCND1* 870A gene variant may be a genetic risk factor in the incidence of advanced cervical cancer.

Keywords Cervical carcinoma · CCND1 · Polymorphism

Introduction

Cervical cancer is one of the most common cancers in women throughout the world [1]. It is responsible for 250,000 deaths per year and approximately 80% of cervical cancer cases emerge in developing regions of Earth [1, 2]. Numerous epidemiological investigations indicate that most cervical carcinomas are etiologically related to oncogenic subtypes of the human papilloma virus (HPV) [3]. Most HPV infections are removed by the host immune system, and only a minority persists and contributes to cervical cancer incidence, which suggests a strong interaction between host factors and the virus [4]. These host factors mainly include genetic components that play a significant role in the susceptibility to incidence of cervical intraepithelial neoplasia and invasive cancer [5, 6]. The genetic impact on cervical cancer incidence varied in different populations, probably due to the effects of environmental factors [5, 7]. However, it has been suggested that approximately 60–70% of the familial risks of cervical cancer include an inheritable component [7].

The *CCND1* gene encodes cyclin D1, which controls the transition from G_1 to the S phase during cell division. Increased levels of cyclin D1 cause premature cell passage through the G_1-S transition, leading to increased spreading of unrepaired DNA damage [8]. This promotes the collection of genetic mistakes and abnormal cell proliferation along with malignant transformation [8, 9]. *CCND1* amplification and increased levels of cyclin D1 have been attributed to different cancer types, providing significant evidence for the oncogenic function of *CCND1* [8].

It has been demonstrated that the *CCND1* A870G transition (rs9344) contributes to the incidence of various cancer types in different ethnic populations [10–18].

This transition produces a silent variant that does not alter the codon 241 proline in the amino acid sequence of cyclin D1 [19]. However, transcription of the *CCND1*-870A variant produces a primary transcript that is alternatively spliced to mRNA designated as transcript b. This transcript is translated to the cyclin D1b isoform, which can be constitutively present in nuclei and can thus exhibit oncogenic properties [20, 21].

The *CCND1* 870A gene variant has been found to be a risk factor for different types of cancer, including cervical

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carcinoma. However, the association of the *CCND1* 870A gene variant with cervical carcinoma incidence has been found to be controversial [16–18, 22, 23]. Therefore, we examined the incidence of the *CCND1* A870G polymorphic variant in patients with cervical cancer (n=129) and healthy individuals (n=288) in a sample of a Polish cohort.

Materials and Methods

Patients and Controls

The patient group comprised of one hundred twenty-nine women with histologically confirmed advanced stage cervical carcinoma according to the International Federation of Gynecology and Obstetrics (FIGO). All patients were disqualified from radical hysterectomy due to advanced stage cancer, and were subjected to radiation therapy between April 2007 and February 2010 at the Department of Radiotherapy, Greater Poland Cancer Center in Poznań, Poland (Table 1). The controls included two hundred eighty-eight unrelated healthy female volunteers who were matched by age to the patients (Table 1). Controls and cases were Caucasians, collected from the same region of Poland. All participating individuals provided written informed consent. The procedures of the study were approved by the Local Ethical Committee of Poznań University of Medical Sciences.

Table 1 Clinical characteristics of patients and controls

Characteristic	Patients n=129	Controls n=288
Mean age±SD	54.9±10.7	55.4±8.2
Tumour stage		
II	25 (19.4%)	
III	95 (73.6%)	
IV	9 (7.0%)	
Histological grade		
G1	9 (7.0%)	
G2	49 (38.0%)	
G3	24 (18.6%)	
Gx	47 (36.4%)	
Histological type		
Squamous cell carcinoma	123 (95.3%)	
Adenocarcinoma	4 (3.1%)	
Other	2 (1.6%)	

Genotyping

DNA was isolated from peripheral leucocytes using a standard salting out process. Identification of the *CCND1* A870G (rs9344) polymorphic variant was conducted by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR was carried out using primer pair 5′ TCTTTCCTTGGTTATGTTTGAGT3′and 5'CCTCCCAGC CAGTCAGTAAG 3'. The PCR-amplified fragments of *CCND1* that were 605 bp in length were isolated and digested with the endonuclease BseNI (ACTGGN/) (New England Biolabs, Ipswich, USA).

The *CCND1* 870A allele was cleaved into 400 bp and 205 bp fragments, whereas the *CCND1* 870G allele remained uncut. DNA fragments were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining. The *CCND1* 870A transition was confirmed by repeated PCR-RFLP assay along with commercial sequencing analysis.

Statistical Analysis

The prevalence of genotypes in patients and controls was examined for deviation from Hardy-Weinberg equilibrium. The chi-square test was used to evaluate differences in genotypic and allelic prevalence between patients and controls. Moreover, the Odds Ratio (OR) and 95% Confidence Intervals (95% CI) were calculated. A p value<0.05 was considered statistically significant.

Results

Genotype analysis of the *CCND1* A870G polymorphism did not show a significant aberration form Hardy-Weinberg equilibrium in control and cases groups. We observed significant differences in the prevalence of the *CCND1* A870G polymorphic variant in patients with cervical carcinoma and healthy individuals (Table 2).

The frequency of the *CCND1* 870AA genotype in patients with cancer and controls amounted to 23% and 17%, respectively (Table 2). Prevalence of the heterozygous *CCND1* 870AG genotype was approximately 1.2-fold higher in patients than in controls, and reached 50% and 43%, respectively (Table 2). We demonstrated that patients with the *CCND1 870*AA and AG genotypes displayed a 1.811-fold increased risk of cervical cancer (95% CI= 1.150–2.852, p=0.0098). However, we did not find significant risk with the homozygous *CCND1* 870AA genotype, OR=1.414 (95% CI=0.8449–2.368, p=0.1858) (Table 2).

To evaluate the association of the *CCND1* 870A allele with cervical cancer, we also studied this allele's prevalence

Table 2 Distribution of G870A polymorphisms in the *CCND1* gene among patients with cervical cancer and healthy individuals

CCND1 G870A (rs9344)	Patients n=129	Controls n=288	OR	95%CI	P[d]
Genotype (frequency)					
G/G	35 (0.27)	116 (0.40)			
G/A	65 (0.50)	123 (0.43)			
A/A	29 (0.23)	49 (0.17)	1.414[a]	0.8449–2.368[a]	0.1858[a]
G/A + A/A	94 (0.73)	172 (0.60)	1.811[b]	1.150–2.852[b]	0.0098[b]
Allele (frequency)					
G	135 (0.52)	355 (0.62)	1.464	1.088–1.969[c]	0.0116[c]
A	123 (0.48)	221 (0.38)			

The Odds ratio was calculated for patients with [a] A/A s vs G/G and G/A genotypes; [b] A/A or G/A vs G/G genotype. We also determined the OR for the patients' minor allele; [c] A allele vs G allele; [d] chi-square test

in both the case and control groups. We found a significantly higher frequency of the *CCND1* 870A allele in patients with advanced cervical cancer than in controls. This allele frequency amounted 48% and 38%, respectively (Table 2). The OR for the *CCND1* 870A allele in patients with advanced cervical cancer was 1.464 (95% CI=1.088–1.969, p=0.0116) (Table 2). We did not observe a significant contribution of *CCND1* A870G genotypes and alleles to cancer characteristics (Table 3).

Discussion

Despite current knowledge indicating that cervical tumors are primarily related to HPV infection, the development and clinical behaviors of this malignancy can be effected by gene variants encoding factors controlling the immune response, metabolic processes, and key regulators of the cell cycle (http://www.hugenavigator.net), [5–7, 24].

Table 3 Prevalence of *CCND1* A870G genotypes between patients tumor stage and histological grade

	Tumor stage			Histological grade		
	II	III	IV	G1	G2	G3
CCND1 G > A						
GG	6	27	2	3	8	9
GA	13	48	4	3	26	13
AA	6	20	3	3	15	2
			P=0.9262			p=0.1101

Data are presented as number, p-values represent significance of genotype distribution between tumor characteristics and were determined by Chi-square test

D-type cyclins (D1, D2, and D3) function as allosteric regulatory molecules for the cyclin-dependent kinases that promote progression during the G1 phase of the cell cycle [25]. D-type cyclins are cyclically biosynthesized during cell cycle progression, and their production and accumulation are associated with the action of extracellular mitogenic factors [26]. It has been reported that cyclin D1 overexpression predominates that of cyclin D2 and D3 in human tumors [8]. Abundant production of cyclin D1 was observed in mantle cell lymphoma, as well as in breast, head and neck, esophageal, and lung cancers [27–31]. Moreover, cyclin D1 is crucial for the neoplastic transformation induced by HPV oncogenic proteins in normal cells, and the subsequent occurrence of cervical cancer [32].

CCND1 overexpression in human cancers can result from numerous mechanisms, including genomic changes, post-transcriptional regulation, and post-translational protein stabilization [8]. The *CCND1* 870A gene variant affects the biosynthesis of the oncogenic variant of cyclin D1 that is persistently located in nuclei [20, 21]. To date, the contribution of the *CCND1* 870A gene variant to the incidence of malignances has been demonstrated in acute lymphoblastic leukemia, as well as in breast, bladder, colorectal, head and neck, esophageal, and cervical cancers [10–18, 33]. However, reports of the association of the *CCND1* A870G polymorphism with various types of cancer, including cervical cancer, have been inconsistent [16–18, 22, 23, 34].

We observed a significant association between the *CCND1* 870A variant and the development of advanced cervical tumors. Our results support a similar investigation in an Indian population, which demonstrated a contribution of the *CCND1* AA genotype to cervical cancer incidence [17]. Moreover, Satinder et al. (2008), showed, in a north Indian population, that individuals with the *CCND1* AA genotype displayed an increased risk of squamous cell

carcinoma of the cervix [18]. Recently, Castro et al. (2009) also found that Swedes bearing the *CCND1* 870A allele displayed a significantly increased risk of cervical cancer development [16]. By contrast, Catarino et al. (2005) showed a significant association of the *CCND1* 870GG genotype with cervical cancer incidence in patients from a Portuguese cohort [23]. The absence of an association between the *CCND1*G870A polymorphism and cervical cancer has been demonstrated in a Korean population [22].

These disparities in the findings of the effect of the *CCND1*G870A polymorphism on the incidence of cervical cancer in various ethnicities may result from differences in the racial heterogeneity of the examined groups. These discrepancies may also be due to each population's exposure to various environmental components, which, along with the *CCND1*G870A polymorphism, may alter the risk of cervical cancer incidence in the investigated ethnicities [7].

The *CCND1* 870A gene variant produces a unique cyclin D1 isoform, cyclin D1b, which lacks the specific phosphorylation site Thr-286. This site is required for nuclear export, and its lack makes cyclin D1b constitutively nuclear [20]. This suggests that subjects with the *CCND1* 870A gene produce the oncogenic cyclin D1b, which preferentially bypasses the G_1-S checkpoint, thereby supporting malignant transformation [20, 21].

It has been demonstrated that cyclin D1 is the downstream target of the neoplastic transformation induced by HPV oncogenic E6/E7 proteins in normal cells [32]. The reduced functionality of the TP-53 and retinoblastoma proteins due to HPV oncogenic proteins can be synergized by the product of the *CCND1* 870A variant [35, 36]. The constitutive nuclear presence of the cyclin D1b isoform may support HPV oncogenic proteins in their disruption of cell cycle checkpoints and promote the initiation of carcinogenesis.

Our findings support the significant role of the *CCND1* 870A gene variant in the development of cervical cancer. However, to more precisely determine the significance of this gene variant in the incidence of cervical cancer, further investigation of this variant's distribution in other sample populations would be valuable.

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