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Transcriptional activation of H- and N-*ras* oncogenes in human cervical cancer

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Abstract

Objective. Overexpression of p21 protein has been detected in human cervical cancer. However, to date, there are no data on the differential activation of the three *ras* genes at the transcriptional level in cervical lesions. The purpose of this study was to evaluate the quantitative and qualitative changes of expression of the *ras* family genes in the development of human cervical cancer.

Methods. The expression of *ras* mRNA levels in 35 human cervical specimens [11 normal cervix, 15 cervical intraepithelial neoplasia (CIN), 9 cervical cancer] was examined using the RT-PCR technique. In addition, we studied the incidence of point mutations in codon 12 of each *ras* gene using RFLP analysis and human papilloma virus (HPV) status.

Results. The transcript levels for H-*ras* and N-*ras* were significantly higher in cancer cases compared to normal cervical tissues ($P = 0.0002$ and $P = 0.001$, respectively) and CIN lesions ($P < 0.0001$ and $P = 0.002$, respectively). The transcript levels for K-*ras* were similar in normal cervical tissue, CIN and cervical cancer. A strong positive correlation was found between H- and N-*ras* expression ($P = 0.001$) and no correlation between H- and K- or N- and K-*ras* expression. Point mutations were detected only in three samples, located in codon 12 of K-*ras* gene. No relationship was found between expression levels of each *ras* gene and the presence of HPV.

Conclusion. Our findings indicate the expression pattern of the three *ras* genes in cervical tissue and the involvement of H- and N-*ras* up-regulation in the pathogenesis of cervical cancer independent of HPV infection.

Keywords: *ras*; RT-PCR; Expression; Cervical cancer; CIN; HPV

Introduction

Cervical cancer is one of the most common malignancies affecting women world-wide. More than 471,000 new cases are diagnosed annually predominantly in less developed countries, where 80% of the world's cervical cancer occurs [1]. In United States, 12,900 new cases are diagnosed each year and approximately one-third of these women die of the disease [2]. In the last decades, the Papanicolaou (Pap) smear screening has led to a steep decline in incidence and mortality from cervical cancer world-wide [3].

Human papilloma virus (HPV) has been identified as the principal etiologic agent for cervical cancer and its precursors. The presence of specific types of HPV—mainly types 16 and 18 ('high risk')—is associated with high-grade intraepithelial lesions and at least 90% of cervical carcinomas [4–7]. The oncogenic potential of HPV is genetically confined to two small open reading frames (ORFs) encoding the proteins E6 and E7 [6,7]. High risk E6 and E7 interact with the host cell DNA and functionally inactivate the p53 and the retinoblastoma (Rb) protein [8,9]. Both p53 and Rb are important tumor suppressor genes, whose products maintain cell cycle regulation, protect the cellular repair process and are involved in apoptosis. To act as oncogenes, however, E6 and E7 have to be uncoupled from their normal function during the regular permissive life cycle [10,11]. In vitro experiments have shown that HPV 16 and 18 are able to immortalize primary keratinocytes, but they are not sufficient to engender a full tumorigenic conversion [12]. Because only a small fraction of HPV-infected women ever develop cervical cancer, additional environmental, lifestyle and genetic factors, such as the activation of cellular

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oncogenes, are likely to be involved in the persistence and progression of HPV infection to cervical cancer.

Harvey (H)-*ras*, Kirsten (K)-*ras* and Neuroblastoma (N)-*ras* genes have been localized to chromosomes 11, 12 and 1, respectively, in humans. All three *ras* genes have a common structure with a 5′ non-coding exon (exon I) and four coding exons (exons I–IV) while the introns of the genes differ widely in size and sequence [13]. The K-*ras* gene has two alternative IV coding exons, thus encoding two proteins, K-*rasA* and K-*rasB*, with K-*rasB* being more abundant. Various reports have been published concerning transcriptional regulation of the H-, K- and N-*ras* genes [13]. All the *ras* oncogenes (H-, K- and N-*ras*) encode for a 21-kDa (p21) protein, 189 amino acids long (except for K-*rasB* which is 188), with GTPase activity which participates in cellular signal transduction [14,15]. Comparison of the amino acid sequence from the three p21 proteins demonstrates complete homology in the N-terminal 86 amino acids, 85% homology in the next 80 amino acids and no homology in the rest.

Activation of *ras* oncogenes by point mutations has been suggested to play an important role in the multistep process of carcinogenesis [16]. The most frequent *ras* alterations in human cancer are mutations in codons 12, 13 and 61 which abolish p21 GTPase activity rendering p21 constitutively activated [16,17]. Overexpression of the *ras* genes has also been shown to confer to carcinogenesis [18]. In vitro experiments have shown that the overproduction of even the normal *ras* p21 protein is sufficient to confer a transforming potential on cultured cells [13,18]. Elevated expression of *ras* genes has been reported for several human cancers including breast, colon, head and neck, bladder and lung and has been associated with the development of the disease [19].

Several studies implicate the activated *ras* genes by point mutations, especially in codon 12, in cervical cancer, although at low frequency [16,20–24]. The *ras* gene mutations have also been detected in non-malignant lesions, indicating that the *ras* genes play an important role in the initial stages of carcinogenesis of the cervix [21]. Elevated expression of *ras* p21 protein has been found in cervical tumors as opposed to benign or premalignant lesions [25–28]. However, expression of *ras* genes in cervix has been studied only at the translational level using immunohistochemical methods. There is no previous data on the transcriptional level of each *ras* oncogene in cervical intraepithelial neoplasia and cancer.

The aim of this study was to examine the expression of N-*ras*, K-*ras* and H-*ras* genes individually at the mRNA level in cervical neoplasia and normal cervix and to investigate their relationship with HPV infection.

Materials and methods

Tissue specimens

Among 77 female patients treated at the Department of Obstetrics and Gynecology, University Hospital of Hera-

Table 1
Primers and PCR amplification conditions used for *ras* expression analysis, detection of codon 12 *ras* mutations and HPV

Gene	Primers	PCR profile
H-*ras* (RNA)	5-GACGGAATATAAGCTGGTGG-3 (S) 5-TAACTACCCCTCTGCACGGA-3 (A)	94°C for 35 s (denaturation) 60°C for 40 s (annealing) 72°C for 40 s (extension)
K-*ras* (RNA)	5-ACTGAATATAAACTTGTGGTAGTTGGACCT-3 (S) 5-CAAATCACATTTATTTCCTACCAGGACCT-3 (A)	94°C for 30 s (denaturation) 58°C for 30 s (annealing) 72°C for 40 s (extension)
N-*ras* (RNA)	5-AATCCAGCTAATCCAGAACC-3 (S) 5-TGGTCTCTCATGGCACTGTA-3 (A)	94°C for 30 s (denaturation) 58°C for 30 s (annealing) 72°C for 40 s (extension)
β-Actin (RNA)	5-AGCCTCGCCTTTGCCGA-3 (S) 5-CTGGTGCCTGGGGCG-3 (A)	
β2-m (DNA)	5-TCCAACATCAACATCCCGGT-3 (S) 5-TCCCCCAAATTCTAAGCAGA-3 (A)	
H-*ras* (DNA)	5-GAGACCCTGTAGGAGGACCC-3 (S) 5-GGGTGCTGAGACGAGGGACT-3 (A)	94°C for 35 s (denaturation) 60°C for 40 s (annealing) 72°C for 40 s (extension)
K-*ras* (DNA)	5-ACTGAATATAAACTTGTGGTAGTTGGACCT-3 (S) 5-TCAAAGAATGGTCCTGGACC-3 (A)	94°C for 35 s (denaturation) 60°C for 40 s (annealing) 72°C for 40 s (extension)
N-*ras* (DNA)	5-AACTGGTGGTGGTTGGACCA-3 (S) 5-ATATTCATCTTACAAAGTGGTCCTGGA-3 (A)	94°C for 35 s (denaturation) 60°C for 40 s (annealing) 72°C for 40 s (extension)
HPV (DNA)	5-TTTGTTACTGTGGTAGATAC-3 (GP5) 5-GAAAAATAAACTGTAAATCA-3 (GP6)	94°C for 50 s (denaturation) 52°C for 45 s (annealing) 72°C for 45 s (extension)

Note: S = sense primer; A = antisense primer.

klion, Crete, Greece from July 2002 to December 2002 who undertook surgical resection or biopsy, we selected for our study all patients with cervical cancer, cervical intraepithelial neoplasia and normal cervix. Finally, our sample group consisted of 11 normal cervical tissues, 15 tissues with cervical intraepithelial neoplasia (6 with CINI, 2 with CINII and 7 with CINIII) and 9 cervical cancers (1 adenocarcinoma and 8 squamous cell carcinoma). Clinical staging was determined using the International Federation of Gynaecology and Obstetrics (FIGO) classification: 2 were IA, 1 was IB, 4 were IIB and 2 were III. Normal cervical tissues used as controls were obtained from patients who had hysterectomies done for non-malignant condition. At the time of surgical resection, the tissue was put in cryotubes and snap frozen in liquid nitrogen. The specimens were subsequently stored at −80 °C. Clinicopathological data (histological subtype, stage, grade, age, tobacco use, lifestyle, outcome and survival data) were available for all specimens examined. The present study was approved by the ethics committee of the University of Crete and all the patients participating in the study gave written informed consent.

Extraction and quantification of mRNA

Total RNA was isolated from fresh tissues using the Trizol reagent (Life Technologies Ltd., UK) according to the manufacturer's instructions. Briefly, 1 ml of reagent was added to each tube. The samples were homogenized using a mechanical mixer and transferred to a 1.5-ml Eppendorf tube. Chloroform (200 μl) was added, and the tube was vortexed and centrifuged at 14,000 rpm for 15 min. The RNA was precipitated with an equal volume of isopropanol and washed with 75% ethanol. The RNA was air-dried and resuspended in water treated with diethyl pyrocarbonate. The RNA preparation was treated with DNase I to remove residual traces of DNA, purified with the phenol–chloroform method and precipitated with ethanol. RNA concentration and purity was determined on a UV spectrophotometer (Hitachi Instruments Inc., USA) by the 260-nm absorbance and 260- to 280-nm absorbance ratio, respectively. One percent agarose gel electrophoresis and ethidium bromide staining were used to examine RNA integrity.

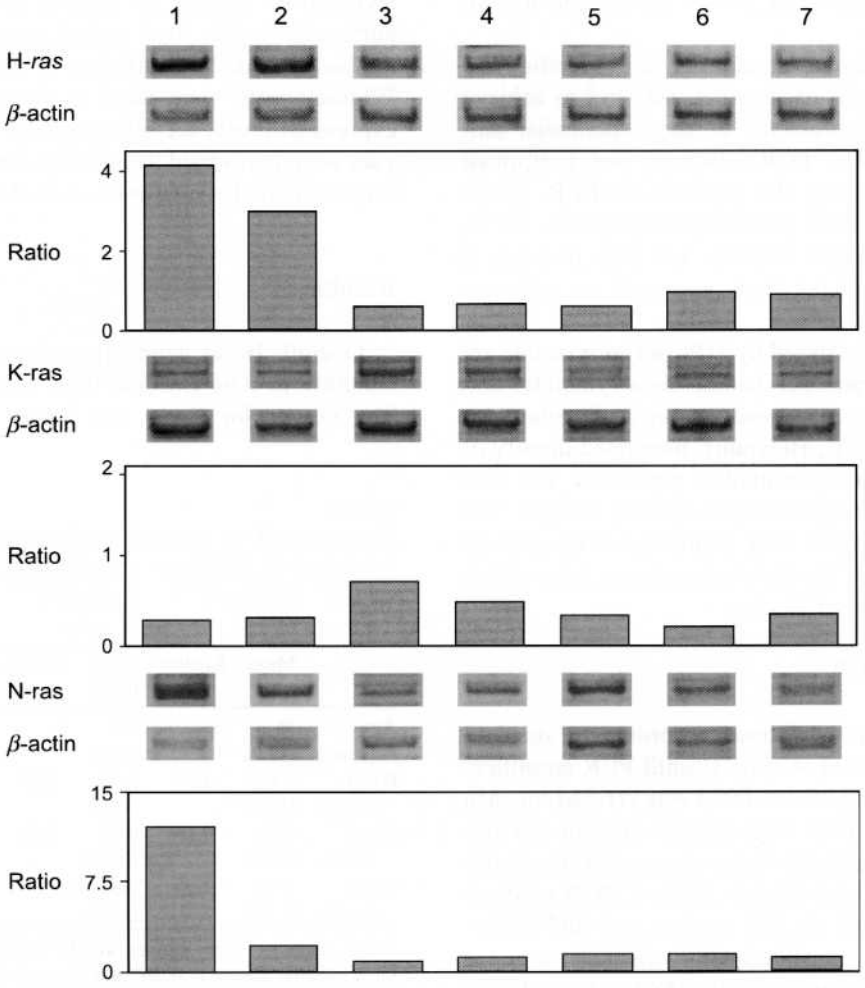

Fig. 1. Representative examples of H-, K- and N-*ras* genes expression in cervical tissue. Samples 1, 2: cancer; 3–5: CIN; 6, 7: normal. Ratio: integrated density of the band of each *ras* gene divided by the integrated density of the band of β-actin.

RT-PCR

Reverse transcription reactions for the preparation of first-strand cDNA from 1 μg of total RNA were performed for 1 h at 52°C, using 15 units Thermoscript reverse transcriptase, 40 units RNaseOut, 50 ng of random hexamers and 1.0 mM of each dNTP in a total volume of 20 μl of 1× First Strand cDNA Synthesis Buffer containing 5 mM dithiothreitol (DTT), ensued by incubation for 20 min at 37°C with 2 units of *Escherichia coli* RNaseH to avoid RNA contamination of cDNA, according to the manufacturer's protocol (Life Technologies Ltd.).

PCR assays were carried out in a PTC-200 programmable thermal controller (MJ Research Inc., USA); 1 μl of cDNA was amplified in a total volume of 10 μl containing 1× PCR reaction buffer, 2.5 mM $MgCl_2$, 0.4 mM dNTPs and 0.6 units Platinum *Taq* DNA polymer*as*e (Life Technologies Ltd.), with 30 pmol of each primer set. The oligonucleotide primers and the amplification conditions used are listed in Table 1. *β-Actin* was used as an internal control in all PCR reactions. The PCR product size of H-*ras* was 151 bp, K-*ras* 357 bp, N-*ras* 150 bp and *β-actin* 175 bp.

To evaluate the above conditions for specific and optimum amplification of each primer set, and to achieve integrated density measurements at the exponential amplification phase of the PCR reaction, we performed kinetic analysis increasing the number of PCR cycles from 24 to 38, for different annealing temperatures 53°C, 55°C and 57°C (data not shown). For this purpose, a mixture of all cDNA preparations was used as reference sample.

PCR products were analysed by 10% polyacrylamide gel electrophoresis (29:1 ratio acrylamide/bis-acrylamide) and silver stained. Gels were scanned on an Agfa SnapScan 1212u (Agfa-Gevaert N.V., Belgium). Integrated density of the bands was used as quantitative parameter and was calculated by digital image analysis (Scion image). The mRNA levels for each gene were expressed as the ratio of the integrated density of the band of each *ras* gene versus the corresponding levels of β-actin.

DNA extraction and PCR

DNA extraction was performed according to standard procedures [21] and stored at −20°C until PCR amplification. The presence of amplifiable DNA was verified for each sample by performing PCR with primers specific for β2-microglobulin. The oligonucleotide primers and the amplification conditions used are listed in Table 1. PCR products for HPV were analysed on 2% agarose gel and photographed on a UV light transilluminator. All PCR reactions included appropriate negative controls. DNA extracted from HeLa cells was used as positive control for the HPV PCR detection method.

RFLP analysis of ras mutations

Twenty microliters of the amplification product was digested for 16 h with 20 units of the restriction endonucleases *Msp*I (H-*ras*) and *Bst*NI (K-*ras* and N-*ras*) in conditions recommended by the suppliers. Ten microliters of the digestion product was electrophoresed through an 8% polyacrylamide gel and silver stained. Genomic DNA from the EJ and SW480 cell lines were used as positive controls for the H-*ras* and K-*ras* codon 12 mutations, respectively [21]. With regard to N-*ras*, genomic DNA from a previously described donor heterozygous for the N-*ras* codon 12 mutation [21] was used as a positive control. Genomic DNA from previously genotyped donors [21] was used as negative controls for all *ras* mutations.

Statistical analyses

The Kruskal–Wallis test was applied to assess possible differences in expression levels of H-, K- and N-*ras* genes between the three patient groups (normal, CIN and cervical cancer) [29]. Pairwise correlations between the expression levels of H- and K-, K- and N-, H- and N-*ras* genes were performed in the total patient group using Kendall's tau method (Kendall's coefficient of rank correlation). Mann–Whitney tests were used to assess differences in gene expression levels according to HPV status. Statistical analyses were performed using SPSS version 11.5. The limit of statistical significance was set at $P < 0.05$.

Results

Overall, H-*ras* gene expression ranged from a ratio of <0.0001 to 9.94 (median 0.95, mean 0.96 and SD 6.87). The ranges for K-*ras* and N-*ras* gene expression were

Table 2
Expression (mRNA levels/mRNA levels β-actin) of H-, K- and N-*ras* family genes in 35 hysterectomy patients[a]

Variable	Group CA (n = 9)		Group CIN (n = 15)		Controls (n = 11)		P-value[b]
	Mean (SD)[c]	Median	Mean (SD)[c]	Median	Mean (SD)[c]	Median	
Age (years)	58 (17.0)	53	48 (14.1)	46	51 (12.0)	49	NS
H-*ras* (ratio)	5.17 (1.59)	4.30	0.43 (11.0)	0.64	0.73 (2.44)	0.91	<0.0001
K-*ras* (ratio)	0.22 (18.2)	0.58	0.14 (20.9)	0.45	0.38 (1.59)	0.36	NS
N-*ras* (ratio)	3.53 (1.96)	2.50	0.87 (12.8)	1.43	1.44 (1.34)	1.29	0.0011

[a] Nine patients with cancer of the uterus (CA), 15 with cervical intraepithelial neoplasia (CIN) and 11 controls.
[b] Kruskal–Wallis tests, NS = not significant ($P > 0.05$).
[c] The mean and SD are presented for H-, K- and N-*ras* ratios, where SD is defined as the antilog transformed SD of the logarithmic values.

Table 3
Detection of H-, K- and N-*ras* mutations in codon 12 and HPV by patient group

Variable	Group CA (n = 9)	Group CIN (n = 15)	Controls (n = 11)
N (%)			
H-*ras* mutation	None	None	None
K-*ras* mutation	2 (22%)	1 (7%)	None
N-*ras* mutation	None	None	None
HPV positive	8 (89%)	11 (73%)	4 (36%)

Table 4
Expression levels of H-, K- and N-*ras* genes by HPV status

Variable	HPV-negative (n = 12)		HPV-positive (n = 23)		P-value[a]
	Mean (SD)[b]	Median	Mean (SD)[b]	Median	
Age (years)	49 (15.4)	48	53 (14.0)	50	NS
H-*ras* (ratio)	0.91 (2.88)	0.93	0.99 (9.72)	0.96	NS
K-*ras* (ratio)	0.24 (11.9)	0.41	0.20 (12.2)	0.45	NS
N-*ras* (ratio)	2.16 (1.98)	1.61	1.19 (8.30)	1.43	NS

[a] Mann–Whitney test, NS = not significant ($P > 0.05$).
[b] The mean and SD are presented for H, K and N ratios, where SD is defined as the antilog transformed SD of the logarithmic values.

$<0.0001-1.25$ (median 0.45, mean 0.21 and SD 11.7) and $0.0001-12.08$ (median 1.55, mean 1.46 and SD 5.87), respectively (Fig. 1). Patient age ranged from 29 to 82 years (median 49 years, mean 51 and SD 14.5 years). There was no evidence of a difference in age between patients with normal cervical tissue, CIN and cervical cancer (Table 2). No significant differences were found in marital status, region of residence, educational level and tobacco use between the three groups.

In Table 3, the *ras* family gene expression ratios are displayed according to patient group. There was strong evidence of a difference in H- ($P < 0.0001$) and N-*ras* ($P = 0.0011$) but not K-*ras* expression between the three patient groups (Table 3). Pairwise analysis indicated that the transcript levels (mean values) for H- and N-*ras* were significantly higher in the cancer cases compared to normal cervical tissues ($P = 0.0002$ and $P = 0.001$, respectively) and CIN lesions ($P < 0.0001$ and $P = 0.002$ respectively) while no significant difference was found between cancer and CIN groups. The highest levels of H- and N-*ras* mRNAs were found in early stage cervical cancer (Stage IA and IB). The transcript levels for K-*ras* were similar in normal cervical tissue, CIN and cervical cancer. We observed a significant positive correlation ($P = 0.001$) between H- and N-*ras* expression (Fig. 2) and no correlation at all between H- and K- or N- and K-*ras* expression in the total sample. H-*ras* appeared to be strongly co-expressed with N-*ras* in both CIN and cancer groups while no correlation was found between H- and N-*ras* expression in normal cervical tissues.

Among the 35 samples analysed, only 3 (2 with cancer and 1 with CIN) were found to contain a K-*ras* mutation in codon 12 (Table 3). No point mutations were found in codon 12 of H- and N-*ras*. No statistical association was found between expression levels of each *ras* gene and the presence of mutation in K-*ras*. As there were only three positive samples with mutations, no correlation with the clinicopathological parameters was undertaken.

HPV DNA was detected in 4 (36%) normal, 11 (73%) CIN and 8 (89%) cervical cancer specimens (Table 3). The highest expression levels of H- and N-*ras* were observed among HPV-positive samples with cancer. However, there was no evidence that expression levels of *ras* family genes differed according to HPV status (Table 4).

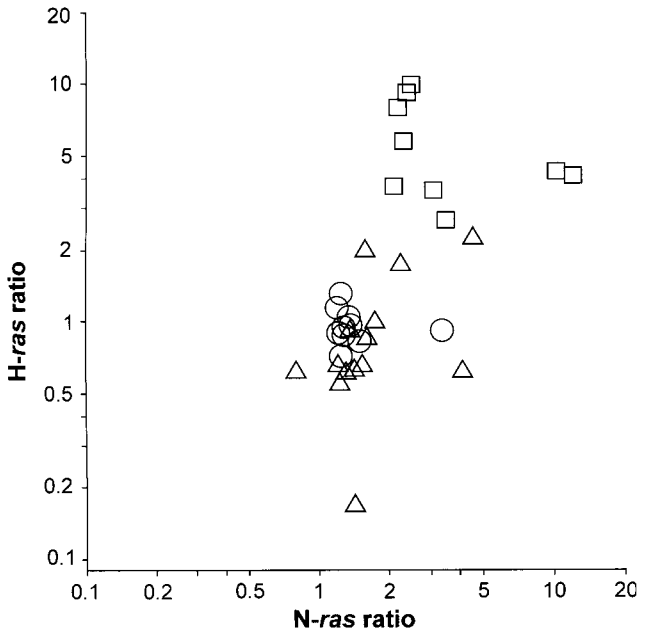

Fig. 2. Relationship between H-*ras* and N-*ras* expression in cervical tissue. □ cancer, △ cervical intraepithelial neoplasia, ○ controls.

Discussion

In the present study, we assessed the transcription levels of H-*ras*, K-*ras* and N-*ras* oncogenes in 35 cervical biopsies using RT-PCR technique. To our knowledge, this is the first report on the differential mRNA expression of the *ras* family genes in human cervical neoplasia. To date, various immunohistochemical studies for cervical cancer and dysplasia have demonstrated p21 immunoreactivity in 12–80% of cases [22,26–28,30]. Elevated p21 protein has been associated with the development of cervical cancer [22] while no differences have been observed between normal cervix and cervical tissue with CIN [25]. Our data demonstrate overexpression of *ras* genes in cervical cancer and suggest that the previously reported high levels of p21 can be attributed specifically to the elevated expression levels of H- and N-*ras* gene.

The frequency of K-*ras* gene mutations at codon 12 in specimens with cervical neoplasia was 3/24 (12.5%) while

no mutations of H- and N-*ras* were detected. This is consistent with the results of previous studies that have shown that the incidence of *ras* mutations in cervical carcinomas is low and preferentially occur at codon 12 of the K-*ras* gene [16,22–24]. In studies focused on the Greek population, the frequency of point mutations at codon 12 of the K-*ras* gene in premalignant and malignant cervical lesions ranges from 17% to 24.4% [20,21]. It has been suggested that the mutational activation of the K-*ras* gene may be involved in the initial stages of cervical carcinogenesis [21].

In our study, we did not find any correlation between expression levels of H- and N-*ras* genes and the presence of mutations in codon 12. Similar results have been reported by Lee et al. [22] who showed no correlation between H-ras protein and codon 12 point mutation in cervical cancer. It appears that other mechanisms, such as amplification and mutation of different codons within the regulatory regions of *ras* genes, may be responsible for protein expression. The relationship between H- and N-*ras* expression suggests the presence of similar regulator elements in the promoter sites of these two genes activated in cervical tissue.

Although the presence of HPV is detected in over 90% of cervical carcinomas [31], it is insufficient to cause carcinogenesis. In vitro studies have demonstrated that activated H-*ras* gene can induce tumorigenic conversion of HPV-immortalized cervical keratinocytes, indicating a cooperative effect between the ras and *E6/E7* genes in cellular transformation [32,33]. It has also been found that overexpression of HPV 18 E6, c-*myc* or activated H-*ras* can partially overcome the growth inhibitory effect of wild-type p53 in NIH3T3 cells [34]. Additionally, expression of oncogenic *ras* induces transformation only in cooperation with another oncogene such as *myc*, E1A, SV40 large T antigen, or when an onco-suppressor gene like *p53* or *p16* is inactivated [35]. In our study, no correlation was found between HPV detection and *ras* expression, indicating that *ras* overexpression may be an important event in cervical tumorigenesis independent of HPV infection. The exact mechanism through which overexpression of *ras* genes is implicated in cervical cancer remains to be elucidated.

The vast majority of low-grade CIN lesions regress spontaneously, and it has been suggested that approximately only 1% of CIN1 lesions and about 10% of CIN3 lesions actually progress to cancer [36,37]. In the absence of genetic markers to monitor disease progression, the current treatment of choice for all high-grade CIN cervical lesions is still complete excision of the lesion inclusive of the transformation zone. Sensitive and objective diagnostic markers that could assess the potential of the invasiveness of cervical neoplastic cells detected in cervical cancer patients would be of tremendous clinical value. The expression of cellular genes such as the ribosomal protein S12 gene has been suggested as early molecular diagnostic identifier for the screening of cervical cancer [38]. In our study, the expression of all *ras* genes was detected in cervical tissues despite the histopathological status. No difference was found in *ras* expression between normal tissues and those demonstrating all grades of CIN. This result is complementary to previous reports [25] using immunohistochemical methods indicating that ras p21 is not likely to be predictive of CIN progression. However, the observed quantitative differences in H- and N-*ras* expression between malignant and non-malignant lesions allow the distinct possibility of employing the *ras* gene family expression as an early molecular diagnostic identifier for the screening of human cervical cancer.

Expression of *ras* p21 has been related to the pathological parameters and the clinical outcome of the patients in a variety of human cancers [17]. In cervical cancer, expression of ras p21 has been associated with poor prognosis [28], lymph node metastasis [39] and occurs in the late stage of the disease [40]. However, observations made by other groups have not supported the significance of *ras* expression in the disease progression and survival [22,30]. In our study, higher levels of H- and N-*ras* mRNAs were found in early stage cervical cancer (Stage IA and IB) than in advanced stage cervical cancer (IIB and III) which is in agreement with observations from related studies on other types of cancer [16]. This finding may indicate a possible role of *ras* expression in the prognosis of cervical cancer because it has been found that the normal H-*ras* can act as an onco-suppressor [41]. The clinical follow-up of our samples will help to examine the significance of *ras* overexpression as a prognostic factor in cervical neoplasia.

Overexpression of *ras* genes can induce neoplastic transformation of cells in vitro [42] and has been associated with the development of several human tumors [43]. Moreover, expression of the oncogenic Ras-family proteins is implicated in enhanced resistance of cancer cells to radiation therapy [44,45]. Karlson et al. [46] have shown that the inhibition of H-*ras* by treatment of a transformed embryonic rat fibroblast cell line with a peptidomimetic FTase-selective inhibitor results in higher levels of apoptosis after radiation therapy. The inhibition of the activated *ras* genes can be achieved with antisense oligonucleotides, ribozymes against *ras* gene products, inhibitors of Ras protein posttranslational modifications and Ras peptide vaccination [13]. On this basis, our data indicate H-*ras* and N-*ras* genes as candidate targets for gene therapy strategies in cervical cancer. To date, adenoviral-mediated transfer of wild-type *p53* or HPV 16 *E6* and *E7* antisense RNA has been associated with cell growth suppression in cervical cancer [47,48]. However, further investigations are warranted to find the complete gene pattern that can be exploited for the molecular treatment of cervical cancer.

The present study provides evidence that H-*ras* and N-*ras* genes are probably involved in cervical carcinogenesis through elevated expression of the normal ras p21 protein. Elevated *ras* gene expression is implicated in the development of cervical cancer independent of HPV status. The precise role of each *ras* gene overexpression to the development of the HPV infection has to be clarified.

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