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Elevated aromatase expression correlates with cervical carcinoma progression

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ABSTRACT

Objectives. We have previously demonstrated that aromatase mRNA is induced in cervical carcinomas compared to normal tissue, suggesting that in situ aromatase expression leading to elevated local estrogen production may contribute to cervical carcinogensis. Our objectives are to examine 1) whether aromatase protein and activity are induced in cervical carcinomas, 2) aromatase expression correlates with disease stage, and 3) inflammatory cytokines (e.g., IL-6 and TNFα) may correlate with aromatase expression.

Methods. RNA and protein were isolated from human cervical carcinomas and normal cervical biopsies to examine aromatase expression, using real-time RT-PCR, Western blot analysis, and immunohistochemistry. Aromatase activity in tissue was measured using the tritiated water release method. IL-6 and TNFα expression was also examined.

Results. Aromatase protein and activity levels were increased in cervical carcinomas compared to normal tissue. RNA levels correlated significantly with disease progression, with highest aromatase expression detected in stage IV tumors ($p<0.001$, $R^2=0.77$). Aromatase promoters 1.3 and 1.4 were elevated in cervical carcinomas and in cervical cancer cells. The expression of inflammatory cytokines IL-6 and TNFα, known to induce aromatase, significantly correlated with aromatase expression ($R^2>0.9$). TNFα treatment induced aromatase expression in cervical cancer cells.

Conclusion. Increased aromatase protein and activity in cervical carcinomas and the correlation of its expression with disease stage implicates it in cervical carcinogenesis. The correlation of IL-6 and TNFα expression with aromatase suggests that these inflammatory cytokines may induce aromatase expression, which is confirmed by induction of aromatase expression due to TNFα treatment of cervical cancer cells.

Introduction

Cervical cancer is the third most common gynecologic cancer and the sixth most common solid malignant neoplasm among women in the United States [1]. In women aged 20 to 39 years, cervical cancer is the second leading cause of cancer deaths, with an estimated 9710 new cases and 3700 deaths from this malignancy [2]. Human papilloma virus (HPV) is a major risk factor for cervical cancer; however, not all HPV positive women develop this malignancy [3,4]. Co-factors like smoking and estrogen are known to contribute to the development of this malignancy [5–8].

Although the etiology of cervical cancer is not completely understood, epidemiological studies have shown that estrogen exposure is a risk factor for cervical carcinogenesis [9,10]. Animal studies have also underscored the importance of estrogen in inducing HPV positive cervical cancer. For example, cervical cancer development in HPV transgenic mice is dependent upon estradiol supplementation [11].

The inflammatory response to HPV viral infection may be a responsible factor in cervical carcinogenesis. The induction of inflammatory cytokines such as IL-6 and TNFα may lead to altered microenvironment conditions and changes in gene expression [12,13]. It is possible that IL-6 and TNFα may induce the expression of aromatase, the enzyme responsible for the last and rate-limiting step in estrogen biosynthesis, leading to increased in situ levels of this steroid hormone.

Aromatase local production has been associated with hormone responsive breast and endometrial carcinogenesis [14–16]. Aromatase expression is regulated by a complex promoter system consisting of 10 exons (e.g., 1.1, 1.2, 1.3, PII, 2a, 1.4 and 1.7) regulated by corresponding promoters. Different cytokines lead to the activation of specific aromatase promoters. For example, TNFα activates aromatase promoter 1.4 [17]. In addition, prostaglandin E2 produced by cyclooxygenase 2 (Cox2) has been shown to activate aromatase promoters PII, 1.3 and 1.7 [18].

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Table 1
Primer sets used for RT-PCR amplification gene primer sequence.

Aromatase	5′AGCATGCTGTACCAGCCTGT3′ (S)
	5′TCATCATCACCATGGCCATGT3′ (AS)
GADPH	5′TGATGACATCAAGAAGGTGGTGAAG3′ (S)
	5′TCCTTGGAGGCCATGTGGGCCAT3′ (AS)
IL6	5′CTTCGGTCCAGTTGCCTTCTC3′ (S)
	5′GCTCTGGCTTGTTCCTCACTACTC3′ (AS)
TNFα	5′GGCTCCACCCTCTCTCCCCTG3′ (S)
	5′TCTCTCAGCTCCACGCCATTG3′ (AS)

We have previously shown that about 35% of cervical carcinomas express aromatase mRNA [8]. Over-expression of aromatase in HPV + ve cervical cancer cells resulted in increased cellular proliferation, anchorage independent growth, ER expression and activity [8]. In the current study we have examined the protein and activity levels of aromatase in cervical carcinoma samples compared to normal cervical tissue. We have correlated aromatase expression with disease stage as well as with the expression of IL-6 and TNFα. Our data suggest that local aromatase induction in cervical tissue may play a critical role in the progression in cervical carcinogenesis and that its expression may be regulated by inflammatory cytokines via the induction of specific aromatase promoters.

Materials and methods

Biopsy and Pap smear samples

Informed consent was obtained as from every patient before being included in the study. The ages of patients ranged from 26 to 85 years with a mean age of 49. Of the 32 cervical carcinoma patients 19 are menstruating. Samples were collected from patients with abnormal

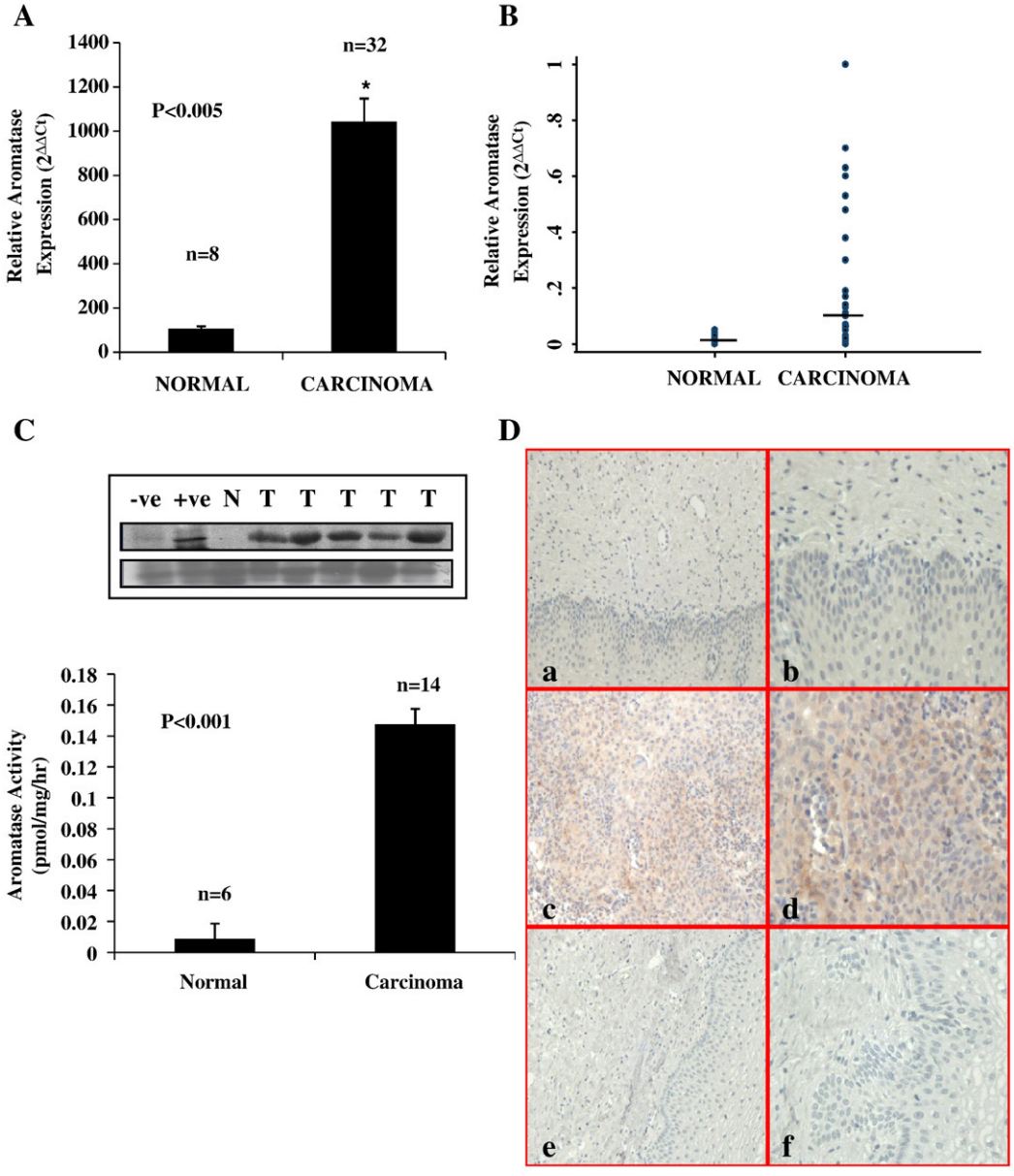

Fig. 1. Aromatase expression is significantly increased in cervical carcinomas. (A and B) aromatase expression was examined in normal ($n=8$) and carcinomas ($n=32$) using real-time RT-PCR. (A) depicts a histogram comparing the mean expression of aromatase in normal tissues to cancer tissues, while (B) is a distribution graph, which shows the actual expression level of aromatase in each sample. 36% of cervical carcinoma samples expressed higher levels of aromatase than the average expression (B). Aromatase expression has been normalized to that of GAPDH. Statistical significance was determined using Student's *t* test ($p<0.005$). Bars, SD. (C) increased aromatase protein levels (inset) and activity was observed. Activity of aromatase enzyme is higher in carcinoma samples ($n=14$) when compared to normal tissue samples ($n=6$). $p<0.001$. (D) aromatase immunohistochemistry analysis of normal (a, b) and cancer tissues (c, d). Negative control without primary antibody staining is shown (e, f).