Human Pathology (2007) **38**, 1226–1231

ELSEVIER

Human PATHOLOGY

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Original contribution

Immunohistochemical study of DNA topoisomerase I, p53, and Ki-67 in uterine carcinosarcomas☆

Sun-Joo Lee MD[a], Hye Sun Kim MD[b], Hy Sook Kim MD, PhD[b,*], Yi Kyung Chun MD[b], Sung Ran Hong MD[b], Je-Ho Lee MD, PhD[c,*]

[a]*Department of Obstetrics and Gynecology, Konkuk University Hospital, Konkuk University School of Medicine, Seoul 143-729, South Korea*
[b]*Department of Pathology, Cheil General Hospital and Women's Healthcare Center, Sungkyunkwan University School of Medicine, Seoul 100-380, South Korea*
[c]*Department of Obstetrics and Gynecology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, South Korea*

Received 1 October 2006; revised 7 January 2007; accepted 11 January 2007

Keywords:
Uterus;
Carcinosarcoma;
DNA topoisomerase I;
p53 protein;
Immunohistochemistry

Summary Uterine carcinosarcomas (UCs) are highly aggressive neoplasms for which no effective adjuvant therapy has been established. The aim of this study was to test potential indicators of UC sensitivity to topoisomerase I (topo I)–targeted drugs. Laboratory studies have shown that the cellular response to topo I–targeted drugs is dependent on topo I expression, DNA replication rate, and activity of the apoptotic pathway. Therefore, this study investigated expression of topo I, a proliferation marker Ki-67, and the apoptosis initiator p53 in 20 cases of UC. Formalin-fixed paraffin-embedded tissue sections were immunostained with monoclonal antibodies against topo I, Ki-67, and p53. The hospital records of all 20 patients with UC were reviewed. Twelve (60%) of 20 cases showed increased expression of topo I. Staining for Ki-67 showed elevated expression in 15 (75%) of 20 cases. Fourteen cases (70%) showed positive staining for p53 in more than 20% of the tumor cells. However, analysis of the relationship between immunohistochemical results and clinical parameters revealed no correlations with topo I expression. There were no significant correlations between the expression of topo I and Ki-67 ($P = .704$), or topo I and p53 ($P = .465$). Significantly increased expression of topo I, Ki-67, and p53 in UC tumor cells suggests sensitivity to topo I–targeted drug treatment.

1. Introduction

Uterine carcinosarcoma (UC) (malignant mixed müllerian tumor) is a rare neoplasm composed of carcinomatous and sarcomatous components. Only about 45% of UCs are confined to the uterine body, and the median survival is 21 months [1]. Histologically, both the epithelial and stromal components of the endometrium are malignant. Although some controversy has arisen over their origin, carcinosarco-

☆ This article was supported by Konkuk University, Seoul, Korea, in 2005.

* Corresponding authors. Je-Ho Lee is to be contacted at Department of Obstetrics and Gynecology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea. Hy Sook Kim is to be contacted at Department of Pathology, Cheil General Hospital & Women's Healthcare Center, Sungkyunkwan University School of Medicine, Seoul, South Korea.

E-mail addresses: hysook.kim@cgh.co.kr (H. S. Kim), jeholee@unitel.co.kr (J.-H. Lee).

0046-8177/$ – see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.humpath.2007.01.012

mas are currently considered to be of monoclonal origin. It also has been suggested that they are metaplastic endometrial carcinomas [2-4]. The operative treatment and staging of UC is identical to that of endometrial carcinoma. Although both radiotherapy and chemotherapy have been used, no effective adjuvant therapy has been established [2]. A Gynecologic Oncology Group phase II study reported that ifosfamide therapy was associated with a total response rate of 34.8% [5]. Paclitaxel has been shown to have moderate activity in patients with UC [6]. However, because advanced or recurrent UC shows poor prognosis and only cisplatin [7] and ifosfamide have demonstrated definitive activity in the UC, the Gynecologic Oncology Group has been performing a series of phase II trials to identify potentially active cytotoxic agents [8].

DNA topoisomerase I (topo I) is the target of several anticancer drugs, including camptothecins (CPTs). Topo I unwinds and uncoils supercoiled DNA by transiently breaking and rejoining single strands of the DNA duplex [9]. CPT and its derivatives stabilize topo I-DNA cleavage complexes and inhibit enzyme catalytic activity by preventing DNA religation. During replication, DNA single-strand breaks are converted into double-strand breaks that are lethal to the cell [10,11]. Several studies suggest that the response of a human tumor to topo I–targeted drugs should at least partly depend on the level of topo I expression [12-15]. In addition, many experimental studies have shown that cellular response also depends on the rate of DNA replication and the activity of the apoptotic pathway [16,17]. Ki-67 is expressed throughout the cell cycle in proliferating cells only. Several studies revealed that elevated expression of Ki-67 has negative prognostic value in various types of human malignant tumors [18]. p53 is a tumor suppressor protein that can induce apoptosis. Mutations of the *p53* gene are the most common genetic alteration observed in many kinds of human neoplasms. Mutant p53 proteins have a longer half-life than the wild-type proteins and are easily detected by immunohistochemical methods. Mutations in the *p53* tumor suppressor gene have several effects on the cell cycle. One well-documented effect of a *p53* mutation is an inability to remain in the G_1 phase of the cell cycle to repair DNA [19]. This suggests that one effect of a *p53* mutation might be an increase in the number of cycling cells in the tumor. p53 causes resistance of cells to topo I inhibitors [20], and the data suggest that *p53* mutated cells are significantly more sensitive to topo I inhibitors than *p53* wild-type cells. In general, p53 overexpression is a marker of aggressive behavior in many kinds of human malignancies and is correlated with poor outcomes [18].

The current study examined the expression of topo I by immunohistochemical staining to determine whether expression levels of the enzyme could predict the efficiency of chemotherapy with CPT and its derivatives in patients with UC. CPT and its derivatives have been shown to be active against several sarcoma and gynecologic cancer cell lines. In addition, we evaluated expression of the proliferation marker Ki-67 because the topo I drug effect is clearly S phase specific and presumably requires active proliferation of tumor cells [21] and expression of p53 to assess the

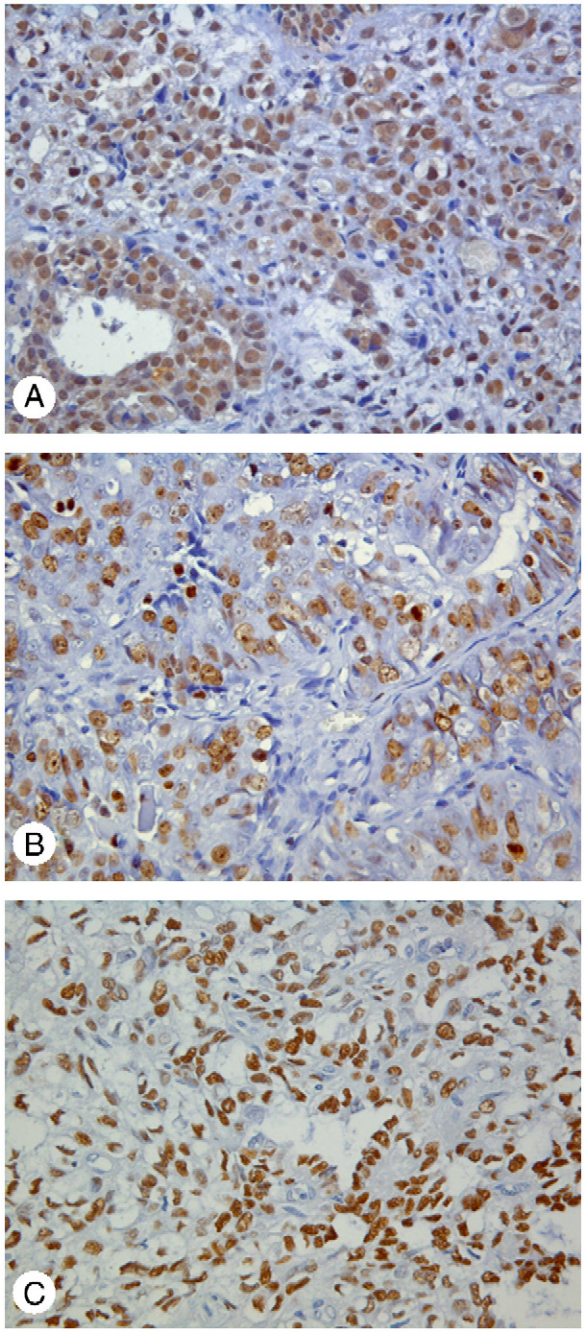

Fig. 1 Immunohistochemical staining for DNA topo I, Ki-67, and p53 in UCs. A, Topoisomerase I immunostaining (original magnification ×400). The carcinomatous component of carcinosarcoma cells was strongly positive and exhibited 3+ staining. B, Ki-67 immunostaining (original magnification ×400). Malignant cells were immunohistochemically stained for Ki-67. The Ki-67 index is 55.5. C, p53 immunostaining (original magnification ×400). Many positive staining cells are present, and the p53 index is 73.3. This was interpreted as positive for a p53 missense mutation.