Development of New Therapeutic Strategies in Gynecological Cancers in Iran by Utilizing Xenograft Model of Ovarian Adenocarcinoma

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Abstract
Objective: To evaluate the potentiality of OVCAR–3 cell line of ovarian adenocarcinoma as a xenograft model for ovarian adenocarcinoma in nude mice. Materials and methods: The cell line isolated from advanced human ovarian adenocarcinoma, were inoculated to eight nude mice and two months later. Established tumors were transferred to pathology laboratory to be prepared by H&E staining and immunohistochemical staining with CA125 antibody. Results: Study of H&E slides showed advanced adenocarcinoma. The CA125 Tumor marker was also positive in tumoral tissue. Conclusion: Established tumors showed excellently the characteristics of ovarian adenocarcinoma. This model can be used to evaluate new treatment strategies. Keywords: Gynecological cancers, Xenograft model, Ovarian adenocarcinoma

Introduction
Ovarian Adenocarcinoma is one of the most important female reproductive cancers and the fifth cause of cancer deaths among women living in the United States (1). Histopathologically, ovarian adenocarcinomas with surface epithelium (Mullerian) origin entail the highest percent of ovarian malignancies which mostly affects 45–65 year old women (2). From the viewpoint of etiology and genetics, several factors are responsible for this cancer's incidence. Mutation in BRCA–1 and BRCA–2 genes causes familial ovarian cancer, while in sporadic ovarian adenocarcinoma cases, structural abnormalities in chromosomes 1 & 11, lack of heterozygosity in 3q, 6q, 11q, 13q, 17q & 17p are of the most important causes of cancer. Also, abnormalities in C–myc, H–ras & neu oncogenes could lead to this disease (3). Since there are no specific clinical symptoms at the early stages of this cancer, therefore, most diagnoses happen at advanced and metastatic stages leading to the poor prognosis (4). Basic research on the aforementioned disease and also using practical cancer modals can result in novel strategies of treat-
ment. The availability of athymic mice (nu/nu) allowed by the mid-1980s the widespread possibility of studying human tumor explants and cell lines grown as xenotransplants. The objective of this study is to establish an ovarian adenocarcinoma xenograft model in Iran. Such a model could give the ability to study the biology of this tumor and assess the efficacy of new chemotherapeutic agents.

Materials and methods

Eight 6-week-old female athymic BALB/c nude mice were utilized in this study. They were housed in filter-capped microisolator cages in the experimental tumor implantation laboratory of Tehran University of medical sciences. Autoclaved water and food were available ad libitum. The experiment was conducted according to ethical principles approved by international animal care and use committees. NIH: OVCAR–3 Cell line isolated from the advanced human ovarian adenocarcinoma, was provided from National Cell Bank of Iran, Pasteur Institute (NCBI) and cultured in RPMI 1640 containing 10 percent FBS. Totally 5×106 cells were inoculated subcutaneously at a 200 µL volume of serum-free medium into the flank of the animals (Fig.1). Two months later mice were sacrificed by CO2 inhalation and obtained tumors after isolating from animal skin were fixed in 10% buffered formalin and sent to the pathology lab. Then one slide was stained with H&E and another slide was stained by immunohistochemistry (IHC) with CA125 tumor marker antibody (DAKO Company) for each tumor, and finally pathologic studies on slides were done.

Results

After subcutaneous SC injection of ovarian cancer cell line to eight female mice, tumors were formed in 3 animals. Pathological study on the slide with staining by H&E showed changes in favor of adenocarcinoma as moderately or poorly differentiated adenocarcinoma and histopathological characteristics of obtained tumors were consistent with the source of standard cell line. Pathological study on the slides stained with CA125 indicated positive result of tumor marker expression in ovarian tumors, which firmly confirmed ovarian adenocarcinoma (Fig. 2).

Discussion

There are no precise statistics showing annual occurrence of ovarian cancer in Iran, however a study performed in 2000–2004, indicates that the 5-year survival between patients diagnosed with ovarian cancer is improved comparing to other countries (5). Also in 2010, a study revealed that the prevalence rate of ovarian cancer in Iran is again much lower in compare to Australia and other developed countries (6). However the total number of occurrence in Iran is 16 per 100000 females. Regarding the fact that in beside of genetic characteristics lifestyle plays an influential role, it is anticipated that in future the number of patients diagnosed with ovarian cancer in Iran will be increased. As a result, the related basic studies must be accelerated.

In 1982, a cell line named NIH: OVCAR–3 was isolated from a female patient suffering from advanced ovarian adenocarcinoma for the first time. In 1984 the xenograft tumor model of this cell line was established (7, 8). This cell line is resistant to Adriamycin and melphan, and expresses androgen and estrogen receptors both in vivo and in vitro (7, 9, 10). Nowadays numerous drugs are evaluated for ovarian cancer treatment using xenograft models. Since tumor marker CA125 is greatly expressed in tumor tissue, it is considered to be quite suitable for further studies. OC125 monoclonal antibodies interacting with MUC16 gene expressing protein CA125, is in progress (11, 12).

Regarding to availability of the nude mice in our experimental laboratory, it seemed that establishment of the xenograft models of ovarian cancer can facilitate the feasibility of basic studies of this cancer. It merits emphasis that Iranian researchers will be able to study the preclinical efficacy of various treatment approaches by means of this xenograft model. Expre-
Development of ovarian cancer xenograft model

Transmission of hormonal receptors in this model will provide a unique opportunity to examination of targeted treatment. On the other hand, due to genetic differences among different ethnic groups, we can isolate native ovarian cancer cell lines and so the sensitivity of chemotherapeutic drugs can be assessed by this ovarian cancer xenograft models in accordance with the pharmacogenomics of Iranians.

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Reference

Figure 2. Immunohistochemical staining with CA–125 stained adenocarcinoma xenograft model tumor marker; (A) ×400 magnification with H&E, (B) ×200 magnification.