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

Zohreh Mazaheri, Ph.D.[2]; Samad Muhammadnejad, DVM–Ph.D.[2]; Ahad Muhammadnejad, DVM–DVSc.[2]; Moein Zargarzadeh, DVM–DVSc.[2]; Maryam Kazem–Haghighi, B.Sc.[2]; Mohammad Ali Oghabian, Ph.D.[3]; Farrokh Tirgari, M.D.[2]; Saeid Amanpour, DVM–DVSc.[1,2]

1 Cancer Research Center, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran
2 Reproductive Health Research Center, Tehran University of Medical Sciences, Tehran, Iran
3 Research Center for Science and Technology in Medicine, Tehran University of Medical Sciences, Tehran, Iran

Received January 2009; Revised and accepted May 2010

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 chromosos 1 & 11, lack of heterozygosity in 3q, 6q, 11q, 13q, 17q & 17p are of the most important causes of cancer. Also, abnormallities 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-

---

Correspondence:
Dr. Saied Amanpour, Reproductive health research center,
Emam Hospital, Keshavarz Blvd., Tehran, 14194, Iran
Tel: +98 (21) 66939320 , Fax: +98 (21) 66581658
E–mail: saiedamanpour@yahoo.com

Figure 1. Xenograft model tumor of humen adenocarcinoma.

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 chemtheraputic 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 CO_2 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 *CA-125* 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 occurence 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 futurethe 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 melaphan, 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-

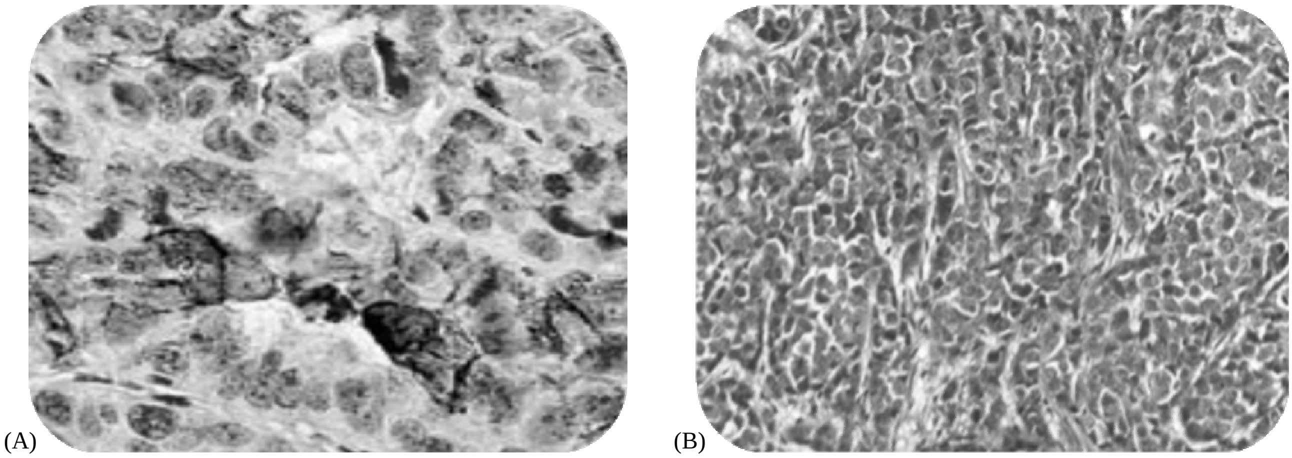

Figure 2. Immunohistochemical staining with CA–125 stained adenocarcinoma xenograft model tumer marker; (A) ×400 magnification with H&E, (B) ×200 magnification.

ssion 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.

Acknowledgement

We sincerely thank the pathology lab of Emam hospital for cooperating in this work.

Reference

1. American Cancer Society. Cancer Prevention & Early Detection Facts & Figures 2009. Atlanta, Ga: American Cancer Society; 2009.
2. Kumar R, Abbas A, Fausto A, Aster D. Robbins and Cotran Pathologic Basis of Disease, Saunders. 8th ed. 2010.
3. Fauci. A, Braunwald. E, Kasper. L, Hauser. J. Harrison's Principles of Internal Medicine. McGraw–Hill Professional. 17th ed. 2008.
4. Jeffrey H. Silber, Paul R. Rosenbaum, Daniel Polsky, Richard N. Ross, Orit Even–Shoshan, J. Sanford Schwartz, Katrina A. Armstrong KA, et al. Does Ovarian Cancer Treatment and Survival Differ by the Specialty Providing Chemotherapy? *Journal of Clinical Oncology* 2007; 25: 1169–75.
5. Arab M, Khayamzadeh M, Mohit M, Hosseini M, Anbiaee R, Tabatabaeefar M, et al. Survival of ovarian cancer in Iran: 2000–2004. Asi*an Pac J Cancer Prev* 2009; 10: 555–8.
6. Arab M, Khayamzadeh M, Tehranian A, Tabatabaeefar M, Hosseini M, Anbiaee R, et al. Incidence rate of ovarian cancer in Iran in comparison with developed countries. *Indian J Cancer* 2010; 47: 322–7.
7. Hamilton TC, Young RC, McKoy WM, Grotzinger KR, Green JA, Chu EW, Whang–Peng J, Rogan AM, Green WR, Ozols RF. Characterization of a human ovarian carcinoma cell line (NIH: OVCAR–3) with androgen and estrogen receptors. *Cancer Res* 1983; 43: 5379–89.
8. Hamilton TC, Young RC, Louie KG, Behrens BC, McKoy WM, Grotzinger KR, et al. Characterization of a xenograft model of human ovarian carcinoma which produces ascites and intraabdominal carcinomatosis in mice. *Cancer Res* 1984; 44: 5286–90.
9. Rogan AM, Hamilton TC, Young RC, Klecker RW Jr, Ozols RF. Reversal of adriamycin resistance by verapamil in human ovarian cancer. *Science* 1984; 224: 994–6.
10. Green JA, Vistica DT, Young RC, Hamilton TC, Rogan AM, Ozols RF. Potentiation of melphalan cytotoxicity in human ovarian cancer cell lines by glutathione depletion. *Cancer Res* 1984; 44: 5427–31.
11. Go Wf. B.A, Hermanto U, Rumbaughl J,Blake J, Bamberg M, Hasan T. Photoimmunotherapy and biodistribution with an OC125–chiorin immunoconjugate in an in vivo murine ovarian cancer model. *Br. J. Cancer* 1994; 70: 474– 80.
12. Duska LR, Hamblin MR, Bamberg MP, Hasan T. Biodistribution of charged F(ab')2 photoimmunoconjugates in a xenograft model of ovarian cancer. *Br J Cancer* 1997; 75: 837–44.