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A HeLa Cell-Implanted Mouse Model of Cervical Cancer

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ABSTRACT

Cervical cancer constitutes the second leading cause of death to heart disease with a prevalence of 100 to 350 in every 100,000 individuals annually. It is a primary cancer of the cervix (cervical canal and/or portio). Carcinogenesis is a somatic event since the accumulation of genetic and epigenetic changes cause changes in the normal regulation of molecular control of cell proliferation. The purpose of the present study was to develop a cervical cancer model by implanting HeLa cells into immunosuppressed mice. True experiments were conducted with 5 DDY mice of 2 months in age and 20–30 gr. in weight obtained from the Integrated Research and Testing Laboratory (LPPT) 4 of Gadjah Mada University. Mice were injected with 0.5 mg/kg of dexamethasone for 7 days to suppress their immunity. Furthermore, 1 ml (8 x 10^6 per microlite) of HeLa cells was injected intracutaneously into the back of the mice. Mice were observed for nodulation at the injection site. Upon reduction of nodule growth, dissection and histopathological examination were carried out with hematoxylin and eosin (H&E) staining. Results showed that the cutaneous tissue of HeLa cell-implanted mice had large-sized cells, greater nuclear volume than that of the cytoplasm and cells losing contact inhibition.
KEYWORDS: Model, cervical cancer, HeLa cells

INTRODUCTION

Cervical cancer is a primary cancer of the cervix (cervical canal and/or portio). Half a million cases are reported annually and the incidence is higher in the developing countries. This is potentially due to non-routine implementation of Pap smear screening programs. In Latin America, sub-Saharan Africa and Southeast Asia, including Indonesia, cervical cancer ranks second after breast cancer. In Indonesia, it was reported the number of new cases of cervical cancer was 100 in 100,000 women per year or 180,000 new cases aged 45 to 54 years. Additionally, cervical cancer tops the list of 10 most common cancers in women. The course of cervical cancer represents one model of multistep carcinogenesis, beginning from early-stage carcinogenesis to changes in morphology to invasive cancers.

HeLa cells can be used to test antitumor activity, tumorigenic transformation, cytotoxicity, cell biology and bacterial invasion. Morphologically, HeLa cells are epithelial cells invaded by the human papilloma virus (HPV) tope 18. The cells are immortal and highly aggressive, making it easy to invade other cell culture/tissue [(1).] Among the grounds for selecting HeLa cells for use in cervical cancer research is that it has the p53 gene that can be induced by the tested compound resulting in cell apoptosis [(2).] Cervical cancer is caused, among others, by infection with Human Papilloma Virus (HPV), leading to abnormal changes in the cervical cells [(3).] Human palpillomavirus is a DNA virus infecting human skin and mucous membranes [(2).] When it infects a cell, the HPV type 18 will express the proteins E6 and E7 [(2,4).] These proteins are suppressor proteins that affect cell proliferation and death. E6 binds to p53 to degrade p53 to prevent cells from undergoing apoptosis, while E7 binds to PRB, leading to cells' continuous proliferation [(2,5).]

The purpose of the present study was to develop a cervical cancer model by implanting HeLa cells into immunosuppressed mice.

METHODS

The present study used the true experiment design with 5 DDY mice of 2 months in age and 20–30 gr. in weight obtained from the Integrated Research and Testing Laboratory (LPPT) 4 of Gadjah Mada University. Mice were injected with 0.5 mg/kg of dexamethasone for 7 days to suppress their immunity.

HeLa cells obtained from the LPPT 4 of Gadjah Mada University were grown from the liquid nitrogen storage with 70% ethanol. Cells were transferred to a sterile conical tube containing the RPMI

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1640 medium. Subsequently, cells were centrifuged at 325 g for 5 min. the pellets were added with the growing medium containing 20% PBS. The cells were then grown in a tissue culture flask and incubated at 37°C in 5% CO_2. The confluent cells were subsequently harvested and washed with PBS without Ca and Mg. Cells were released by adding 0.25% trypsin and then 10 ml of RPMI 1640. Cells were centrifuged for 5 min. Furthermore, cells were added with the growing medium 20% PBS to obtain a concentration of 8 x 10^6/100 microlites.

The medium used in the HeLa cell culture was RPMI 1640-serum since it contains nutrients required by the cells such as amino acids, vitamins, organic salts and glucose, while serum contains hormones that enhance cell growth. Albumin serves as transport protein, lipids as cell growth support and minerals as enzyme cofactor. All the components of RPMI-serum media are to provide the cells with sufficient nutrients in order to survive and proliferate [(6).]

Furthermore, 1 ml (8 x 10^6 per microlite) of HeLa cells was injected intracutaneously into the back of the mice. Mice were observed for nodulation at the injection site. Upon reduction of nodule growth, dissection and histopathological examination were carried out with hematoxylin and eosin (H&E) staining.

Ethics

Care of animal subjects and experimental procedure was approved by Research Ethics Committee of Airlannga University Medical School, Malang, East Java, Indonesia.

RESULTS

1. Cervical cancer induction of HeLa cell-implanted mice

Cancer in the present study was induced by implanting HeLa cells into the back of immunosuppressed mice. Implantation was carried out by injecting 8 x 10^6 HeLa cells per microlite. Results showed 100% of the mice developed nodules in their skin. Induction of cancer produced local and unmetastatic tumors. Intracutaneous implantation of HeLa cells were aimed at maintaining the cells in place. This situation allowed for nodulation at the implantation site.

The formed nodules were initially soft in consistency since it contained HeLa cell suspension but it then changed to be hard in consistency on day 3 of implantation.

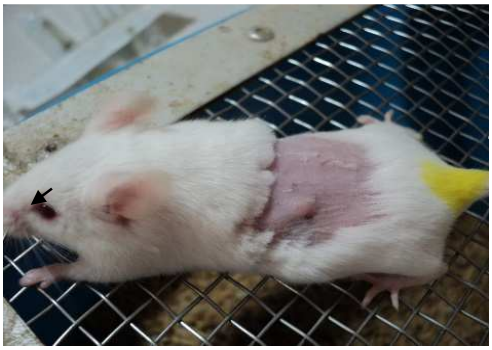

Figure 1. Nodules produced by implantation of HeLa cells into the back of mice. It appeared on day 3 of intracutaneous implantation and was hard in consistency.

The growth of nodules was observed daily for 12 days. Nodules began to appear on day 3 of implantation. It reached the optimum value on day 8 and then began to decline on day 9.

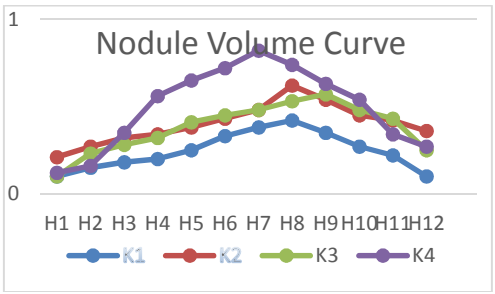

Figure 2. The curve of nodule volume produced by implantation of HeLa cells.

2. Microscopic features of HeLa cells implanted into mice

Results showed that the cutaneous tissue of HeLa cell-implanted mice had large-sized cells, greater nuclear volume than that of the cytoplasm and cells losing contact inhibition. HeLa cells implanted into the cutaneous tissue could not form massive tumors. The features of HeLa cells implanted into the cutaneous tissue resembled the vaginal cytologic smears of patients with cervical cancer, in which the cells have been washed away from the surface of the tumor or aspirated from the mass through a fine needle.

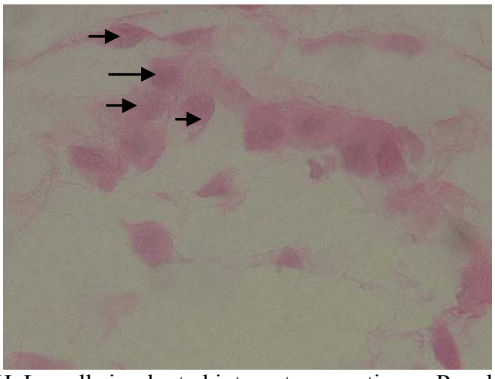

Figure 3. The features of HeLa cells implanted into cutaneous tissue. Results of H&E staining (1000x magnification) showed cells of similar size with the nuclear volume larger than that of cytoplasm. The cells were not adherent to each other.

DISCUSSION

A cervical cancer modeling by intra-epithelial implantation of 1.5 x 10^6 HeLa cells into the skin tissue was made by Marquez-Lemus *et al*. [(6)] into the legs of female nu/nu mice of 3–4 weeks in age. It was capable of producing tumors appearing on day 15 of implantation without metastasis. The nu/nu (nude) mice are genetically mutated mice to have no thymic glands. Thus, they cannot produce T cells (lymphocytes) that play an important role in the immune system, leading to incapability to generate an immune response. Therefore, nude mice cannot reject a xenograft, or a tissue transplant from other species.

The present study used DDY mice of 2 months in age and 20-30 gr. in weight. Mice were intramuscularly (IM) injected with 0.5 mg/kg B.W. of dexamethasone into their thigh for 7 days aimed at depressing their immune system. Dexamethasone is a steroid that depresses the immune system. However, it should not be used excessively since it can damage the immune system of mice, leading to death. Of 5 mice used in the study, one was dead after dexamethasone injection. The success rate of the immunosuppression technique used in the study was 100%, as evidenced by the mice's appearing relatively weak and losing appetite after dexamethasone injection. However, the immunosuppression was also temporary; thus, naturally the formed nodules would shrink and eventually disappeared.

One of the forms of immune response to the implantation of cells is inflammation. In the implantation site, the pre-capilary arterioles will be dilated and the post-capillary venules will be narrowed, thereby increasing the local blood flow. These events may cause swelling and redness typical of inflammation[(7)]. Swelling and redness can be clearly observed after isolation of nodules.

Inflammatory responses are initiated by the presence of chemical signals. The chemical signals derive from the invading organisms/cells. The chemical signals are pro-inflammatory cytokines, such as histamine and serotonin. Histamine produced by circulating leukocytes, called basophils, and mast cells can be found in the connective tissues. In the event of a lesion, these cells stimulate the release of histamine and trigger enlargement and increase in capillary permeability. Leukocytes and cells of damaged tissues secrete prostaglandins which in turn will increase blood flow to the injured site. An increase in local blood flow and capillary permeability will increase macrophage migration to the injured tissue. Furthermore, macrophages, along with neutrophils, will phagocytize dead (necrotic) tissues [(7).]

Nodules formed after implantation of HeLa cells contain tumor cells and cells produced by immune reaction. Results of the present study showed that the optimum termination was observed on days 7 and 8 of implantation. This showed that the cells have been subjected to inflammatory process due to stimulation of HeLa cells implanted. Implantation of HeLa cells into the cutaneous tissue caused

infiltration of neutrophils to the implantation site, resulting in swelling in the cutaneous layer. A proper intracutaneous implantation was shown by the presence of cells in the cutaneous layer.

Cancer cells have a morphology different from that of normal cells [8]. Thus, hematoxylin and eosin (H & E) staining is required on the emerging nodules. In the present study, examination of HeLa cell- implanted skin tissue (nodules) by the use of the H & E staining method indicated large-sized cells with pink nuclei and nuclear volume larger than that of the cytoplasm. Cells were not adherent to one another (loss of contact inhibition). HeLa cells implanted into the citaneous tissue could not form massive tumors.

The features of HeLa cells implanted into the cutaneous tissue resembled the vaginal cytologic smears of patients with cervical cancer, in which the cells have been washed away from the surface of the tumor or aspirated from the mass through a fine needle [8]. Tumor cells have a large prominent nucleus of irregular shape and few cytoplasms [9].

CONCLUSION

The features of HeLa cells implanted into the cutaneous tissue resembled the vaginal cytologic smears of patients with cervical cancer, in which the cells have been washed away from the surface of the tumor or aspirated from the mass through a fine needle.

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