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Mini Review

Common Orthotopic Establishment of Four Cancer-Bearing Mouse Models

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1. Abstract

Cancer is a major public health problem worldwide. Although cancer death rates have been continuously declining for the past 2 decades, it is currently the second and first leading cause of death in the United States and Taiwan, respectively. Cancer is expected to surpass heart diseases as the leading cause of death in the next few years. The top 10 cancer types for estimated cases and deaths worldwide in 2018 were presented that lung cancer is the most commonly diagnosed cancer and the leading cause of cancer death, closely followed by female breast cancer, colorectal cancer, and prostate cancer for incidence and colorectal cancer, stomach cancer, and liver cancer for mortality. Therefore, the establishment of the suitable cancer-bearing animal models and therapeutic strategies for development of more effective treatments for inhibition, not only of proliferation, but also of cancer metastasis is urgently needed. In this study, we presented the successful establishment of four orthotopic xenograft or allograft cancer-bearing mouse models. We hope these cancer-bearing mouse models will applied to the new anti-cancer drug research and development in the future.

2. Keywords

Cancer-bearing models; Establishment; Mice; Orthotopic implantation

3. Brief Introduction of Cancer Prevalence in the World and Taiwan

The incidence and mortality of cancer are rapidly growing worldwide. The reasons are complex and both aging and growth of the population are related with the prevalence and distribution of the main risk factors for cancer. With rapid population growth and aging worldwide, the rising prominence of cancer as a leading cause of death partly reflects in mortality rates of stroke and coronary heart disease, relative to cancer in many countries. According to the reports of the World Health Organization (WHO) in 2015, cancer is the first or second leading cause of death before age 70 years in 91 of 172 countries. In 2019, WHO lists cancer as one of top 10 threats to public health. Nearly, 10 million people die of cancer each year worldwide. Additionally, some reports presented that the global cancer cases will be increase to 60% in 2040. There may be nearly 29.4 million new cases of cancer each year [1].

The important causes of cancer include living habits, genes, and environment etc. The incidence rates vary widely, and even the prevalent cancer types are often very different in the world. Cancer is also a threat to the lives of people in Taiwan. According to the statistics of Taiwan's government, cancer has been the top 10 cause of death in Taiwan for many consecutive years since 1982. According to the latest 2018 reports of Taiwan's government, the top 5 cancers among Taiwanese are lung cancer, liver cancer, colorectal cancer, female breast cancer, and oral cancer. According to the latest global cancer incidence rate published by the Organization for Economic Cooperation and Development (OECD), Taiwan's cancer incidence rate is 296.7 per 100,000 population, ranking 10th among 45 countries in the world. Other 9 countries include Denmark, Australia, Belgium, Norway, United States, Ireland, South Korea,

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Netherlands, and France. In recent years, the threat of colorectal cancer to people's health has also increased day by day, and it is no more than women with breast cancer in Taiwan. The colorectal cancer's favorability for men and women is obviously regardless of gender [1, 2].

4. Murine Tumor Models

At present, many murine tumor models have been reported [3-5]. These murine tumor models included as syngeneic tumor models (STM), genetically engineered models (GEM), patient-derived xenograft models (PdXM), and humanized patient-derived xenograft models (HPdXM). STM are the oldest and most heavily utilized preclinical models to evaluate anticancer therapeutics. C57BL/6, BALB/c, and FVB mice are often use to establish cancer-bearing models via the isolated spontaneous, carcinogen-induced, or transgenic tumor cell lines. GEM predominantly utilize either tissue-specific promoters to drive either expression of an oncogene or to drive deletion of tumor suppressors. It is possible to not only drive autochthonous invasive cancer development but also develop precancerous lesions by using these genomic alterations. Human xenograft models (PdXM and HPdXM) were via using human cell lines injected into immunodeficiency animals. These mod- els can be utilized either human cell lines or patient-derived sam- ples to generate xenografts for antitumor efficacy evaluation [3-8].

The advantages and disadvantages are found in these caner-bearing models as the advantages in the STM include reproducible and rapid growth, easily manipulated, and no host breeding requirements. Disadvantages in the STM include the lack of native tumor microenvironment, variability of phenotype depending on the site of engraftment, relatively few transplantable cell lines, the lack of heterogeneity, and relatively few host strains; the advantages in the GEM include the autochthonous growth provides native microenvironment, tumor development driven by relevant genetic alterations, and can incorporate genomic instability. Disadvantages in the GEM include the breeding challenges, variability in penetrance and latency, and low immunogenicity due to defined perturbations; the advantages in the PdXM include reproduces complexity of human disease and does not require immune reconstitution. Disadvantages in the PdXM include conducted in immunodeficient host, murine stroma, and low implantation rate; the advantages in the HPdXM include the reproduction of complexity of human disease and immune system and can utilize engineered hosts to increase immune reconstitution. Disadvantages in the HPdXM include requires autologous immune reconstitution and low rates and duration of immune reconstitution [3, 9-13].

5. Establishment of the Orthotopic Cancer-Bearing Models in Mice in Laboratory

Establishment of the orthotopic xenograft cervical cancer model, the orthotopic allograft prostate cancer model, the orthotopic xenograft ovarian cancer model, and the orthotopic allograft colorectal cancer model are often performed in laboratory. The protocols of these models are as female/male NOD SCID mice (NOD.CB17-*Prkdc*^{scid}/JNarl), C57BL/6 mice (C57BL/6JNarl), BALB/c nude mice (BALB/cAnN.Cg-*Foxn1*^{nu}/CrlNarl), and BALB/c mice (BALB/cByJNarl), aged 8 weeks, were respectively anaesthetized by 0.4% isofluorane inhalation and the target organs as uterus, prostate gland, ovarian, and colon were respectively exposed by an aseptic incision. Then, cancer cells as HeLa cells (ATCC® CCL-2™; 5×10^6 cells in 5 μL DPBS), TRAMP-C1 cells (ATCC® CRL-2730™; 1×10^5 cells in 10 μL DPBS), SK-OV-3 cells (ATCC® HTB-77™; 2.0×10^6 cells in 3 μL DPBS), and CT26 cells (ATCC® CRL-2638™; 1×10^7 cells in 10 μL DPBS) were respectively injected into the target organs by an Hamilton syringe (30G). The wounds on the dorsal/ventral abdomen and skin were closed in two layers using 4-0 silk sutures. Finally, after four or ten-week cancer cell implantation, cancer-bearing mice were sacrificed and measured the size and weight of the tumor (Figure 1-4).

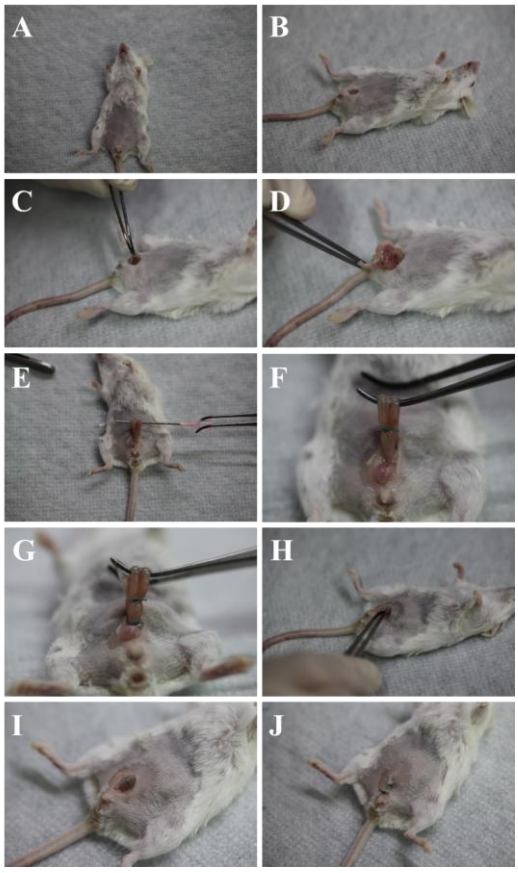

Figure 1: Establishment of the orthotopic xenograft cervical cancer model. (A) Eight-week-old female NOD SCID mice (NOD.CB17-*Prkdc*^{scid}/JNarl) were anaesthetized; (B) Lower abdominal midline incision; (C-E) The uterus was exposed; (F-G) The bottom of uterine horn and vagina were tied by using 4-0 bioabsorbable PGA sutures; (H) HeLa cells (ATCC® CCL-2™; 5×10^6 cells in 5 μL DPBS) were injected by an Hamilton syringe (30G) through the anterior vaginal fornix into the space between the anterior vaginal fornix and the anterior wall of the cervix; (I) The wounds on the abdomen and skin were closed in two layers using 4-0 silk sutures. Finally, after four weeks tumor cell implantation, tumor-bearing mice were sacrificed and measured the size and weight of the tumor.

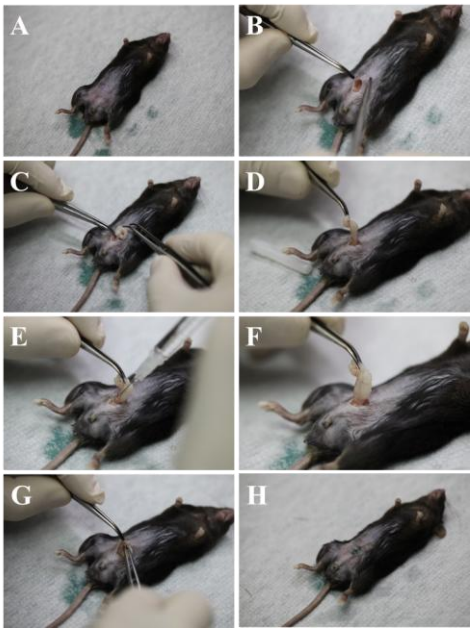

Figure 2: Establishment of the orthotopic allograft prostate cancer model. (A) Eight-week-old male C57BL/6 mice (C57BL/6JNarl) were anaesthetized; (B) Lower abdominal midline incision; (C-D) The prostate gland was exposed; (E-F) TRAMP-C1 cells (ATCC® CRL-2730™; 1×10^5 cells in 10 μL DPBS) were injected by an Hamilton syringe (30G) through the anterior prostate gland; (G-H) The wounds on the abdomen and skin were closed in two layers using 4-0 silk sutures. Finally, after ten weeks tumor cell implantation, tumor-bearing mice were sacrificed and measured the size and weight of the tumor.

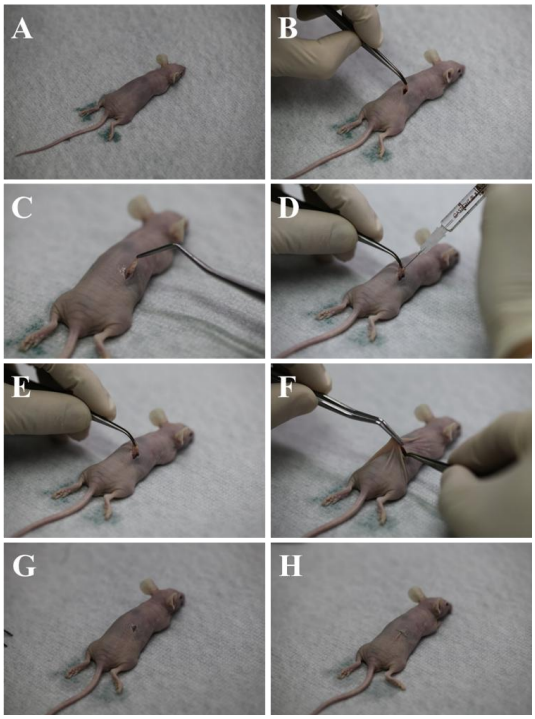

Figure 3: Establishment of the orthotopic xenograft ovarian cancer model. (A) Eight-week-old female BALB/c nude mice (BALB/cAnN. Cg-*Foxn1*^{nu}/CrlNarl) were anaesthetized; (B) Right dorsal midline incision; (C) The ovary was exposed; (D-E) SK-OV-3 cells (ATCC® HTB-77™; 2.0×10^6 cells in 3 μL DPBS) were injected into ovarian bursa by an Hamilton syringe (30G); (F-H) The wounds on the dorsal abdomen and skin were closed in two layers using 4-0 silk sutures. Finally, after 4 weeks tumor cell implantation, tumor-bearing mice were sacrificed and measured the size and weight of the tumor.

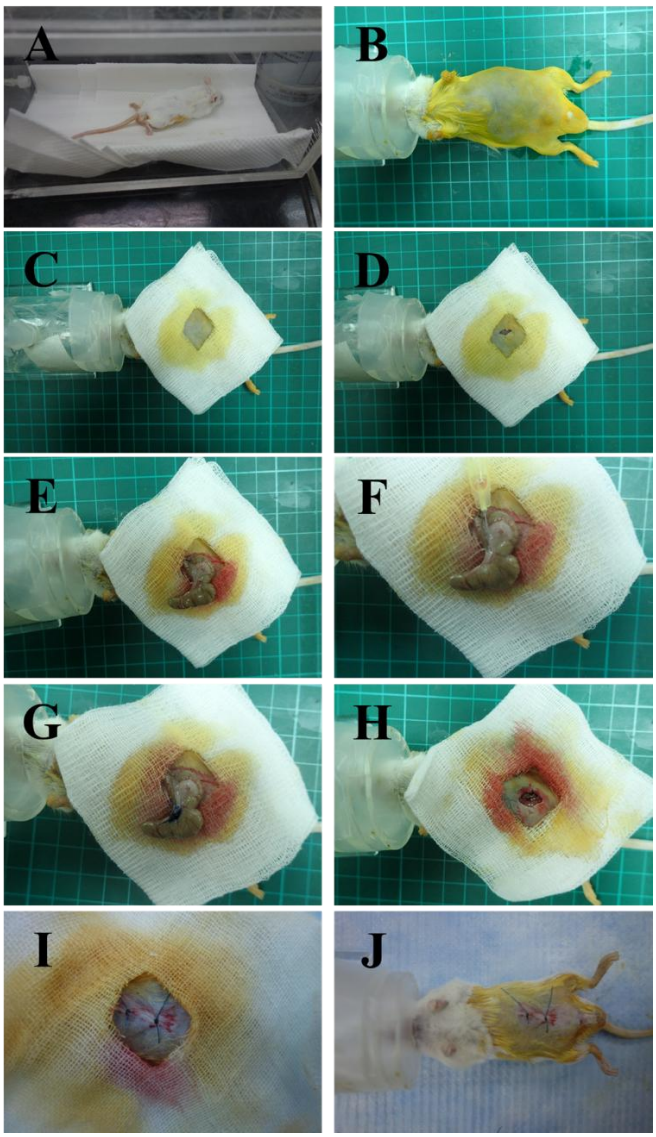

Figure 4: Establishment of the orthotopic allograft colorectal cancer model. (A-C) Eight-week-old male BALB/c mice (BALB/cByJNarl) were anaesthetized; (D) Abdominal midline incision; (E) The colon was exposed; (F) CT26 cells (ATCC® CRL-2638™; 1×10^7 cells in 10 μL DPBS) were injected into the interspace of the serosa and mucosa of colon by an Hamilton syringe (30G); (G) Ten μL trypan blue as demonstration was injected into the interspace of the serosa and mucosa of colon by an Hamilton syringe (30G); (H-J) The wounds on the abdomen and skin were closed in two layers using 4-0 silk sutures. Finally, after 4 weeks tumor cell implantation, tumor-bearing mice were sacrificed and measured the size and weight of the tumor.

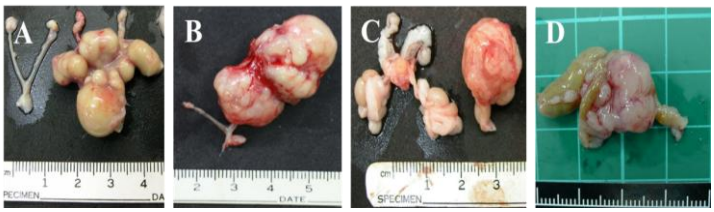

Figure 5: Orthotopic implantation of tumor cell lines via aseptic surgery and micro-injection. (A) Cervical cancer; (B) Ovarian cancer; (C) Prostate cancer; (D) Colorectal cancer.

6. Future Perspectives

Recently, FDA has approved cancer immunotherapy. The development of effective preclinical cancer-bearing animal models to reliably elucidate anticancer mechanism and predict anticancer efficacy remains an area of critical unmet need. In addition, the adverse events under therapeutic strategies will be a critical application of preclinical cancer-bearing animal models [3, 14-20].

7. Conclusion

Cancer is a major public health problem worldwide. Although these patients with cancer may be removed surgically and chemotherapy, paclitaxel, cis-platinum analogues, and doxorubicin, has generally been adopted after surgery. However, cancer is expected to surpass heart diseases as the leading cause of death in the next few years. Therefore, the establishment of the suitable cancer-bearing animal models for research and development (R&D) of more effective treatments as novel drugs and therapeutic strategies for inhibition, not only of proliferation, but also of cancer metastasis is urgently needed. In this study, we presented the successful establishment of four orthotopic cancer mouse models (prostate, cervical, ovarian, and colorectal cancer). We hope that theses cancer-bearing mouse models will be applied to R&D of the new anti-cancer drugs in the future.

8. Acknowledgements

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9. Conflicts of interest

The authors declare no conflict of interest.

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