Novel bone microenvironment model of prostate cancer with chitosan fiber matrix and osteoblast in 3D culture.

Masahiro Samoto[1)] , Hiroaki Matsumoto[1)] , Hiroshi Hirata[1)] , Koji Ueno[2)] , Sho Ozawa[1)] , Junichi Mori[1)] , Ryo Inoue[1)] , Seiji Yano[1)] , Yoshiaki Yamamoto[1)] and Hideyasu Matsuyama[1)]

1)Department of Urology, Graduate School of Medicine, Yamaguchi University 2)Center for Regenerative Medicine, Yamaguchi University Graduate School of Medicine

BACKGROUND

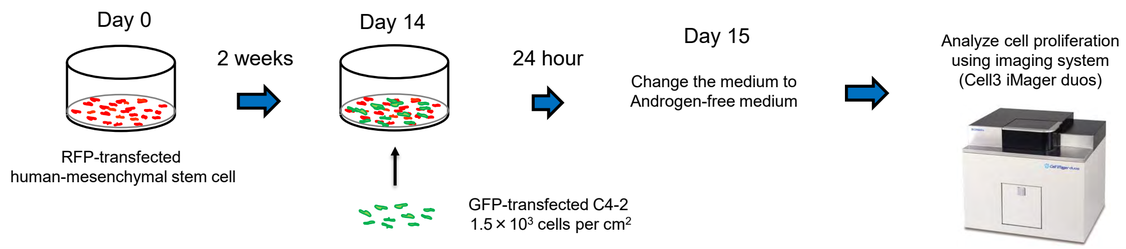
The interaction between prostate cancer cells and osteoblast is essential for the development of bone metastasis. Recently, novel androgen receptor-axis-targeted agents (ARATs) have been approved for metastatic castration naïve prostate cancer (mCNPC), or non-metastatic castration resistant prostate cancer (nmCRPC), both of which should be pivotal to investigate the association between bone microenvironment and tumor. We established a novel 3D in vitro culture method reflecting bone microenvironment and evaluated the drug susceptibility of ARATs including enzalutamide, apalutamide, darolutamide, abiraterone (Abi) with/without dutasteride (Duta).

METHODS

1. Bone microenvironment model
RFP-transfected human-mesenchymal stem cells were plated (5×10^4 cells/cm^2) onto the chitosan nanofiber coated culture plate, and incubated with MSC Growth Medium2 (Promo cell). When cells reach at confluency, medium was changed to MSC Osteogenic Differentiation medium (Promo cell)(Day0), followed by incubation for 14 days to induce human osteoblast. On day 14, GFP-transferred C4-2 (CRPC cell line, 1.5×10^3 cells/cm^2) was added and co-cultured with human osteoblast. On day 15, the medium was changed to androgen-free medium, phenol red-free RPMI 1640 medium supplemented with 5% charcoal treated fetal bovine serum. The growth of C4-2 and Osteoblast were quantified using imaging system (Cell3 iMager duos , SCREEN, Tokyo) every 3-4 day.

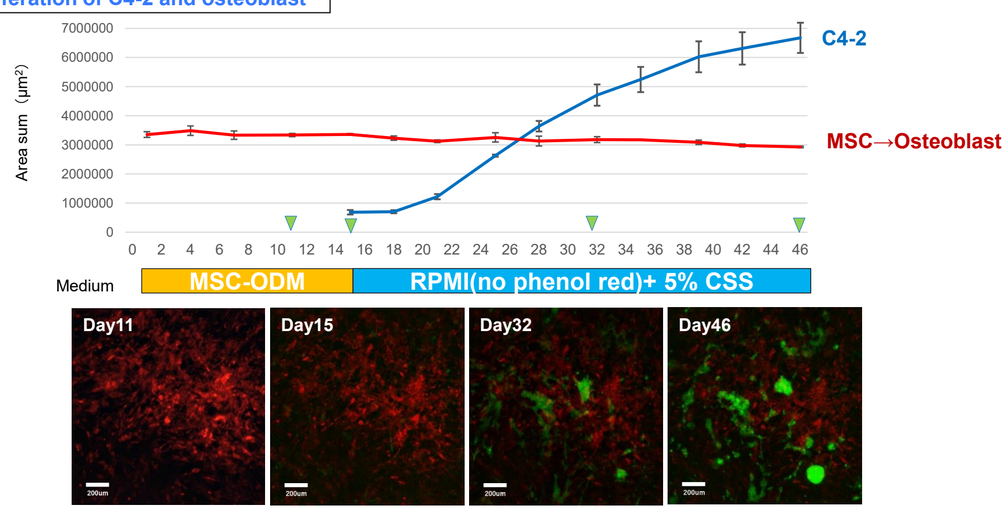
2. Drug sensitivity testing
Totally 4 ARATs (Enzalutamide, Aparutamide, Darolutamide, and Abiraterone) were selected for drug sensitivity test using microenvironment model. Each drugs were added to culture medium at a concentration of 5uM dissolved by ethanol on day 15. The effect of drug was evaluated as average of green intensity area of well.

3. Statistical test
To compare the statistical difference of colony growth inhibition, Student's t-test was applied.



RESULTS

Fig.1 Proliferation of C4-2 and osteoblast



Long-term survival of osteoblasts and long-term proliferation of tumor cells were observed in the chitosan nanofiber coated 3D culture. C4-2 formed colonies.

Fig.2-A Comparison of colony growth between 2D culture and 3D culture

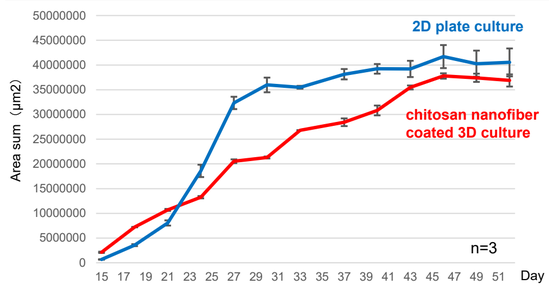
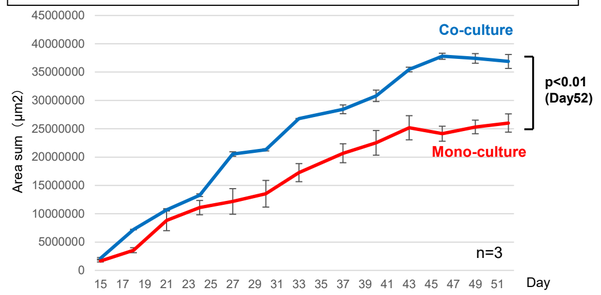


Fig.2-B Comparison of colony growth between mono-culture and co-culture with osteoblast



A) C4-2 and human osteoblast was co-cultured in chitosan nanofiber 3D culture , and in 2D plate culture with the same culture methods. Prolonged growth was observed when C4-2 was co-cultured with osteoblasts in 3D culture.
B) Compare the growth of C4-2 in Mono-culture and Co-culture with human osteoblast in chitosan nanofiber coated 3D culture plate. Co-culture with human osteoblast showed growth enhancement effect of C4-2.

Fig.2-C Drug sensitivity testing among ARATs

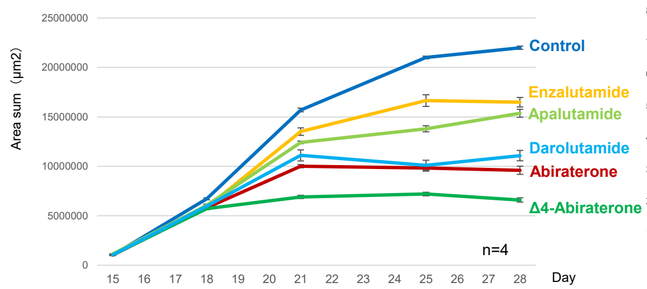
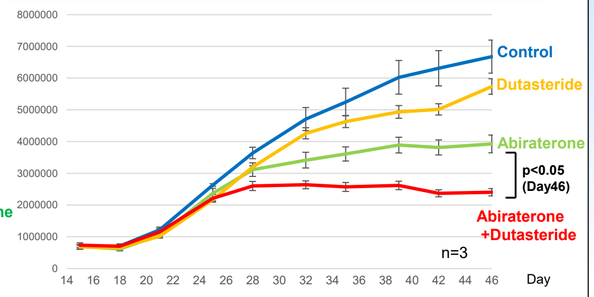
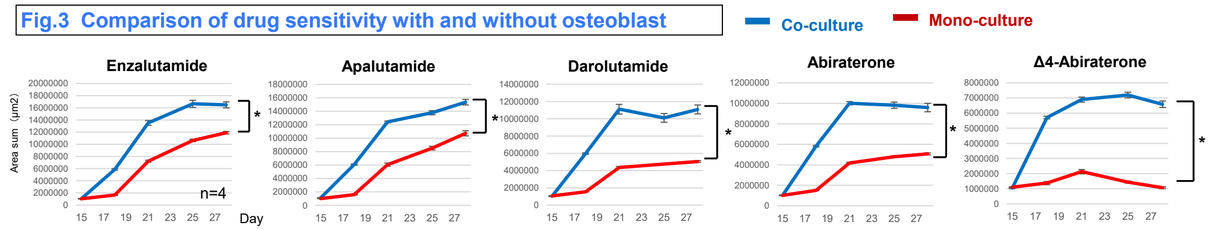


Fig.2-D Comparison of Colony inhibition among Abiraterone, Dutasteride, and both combination



C) Differences in C4-2 growth were observed with added ARATs drugs.
D) Combination of Abiraterone and Dutasteride showed the potent growth inhibitory effect in this model.

Fig.3 Comparison of drug sensitivity with and without osteoblast



Compare the growth of C4-2 in Mono-culture and Co-culture with human osteoblast, each drugs were added to culture medium. Co-culture with human osteoblast reduce growth inhibitory effect in all drugs. (* p value <0.01)

CONCLUSION

1. **We could quantify the sustained growth of C4-2 cells at maximum of 35 days in bone-microenvironment model.**
2. **Each drugs and combination effect of Abi and Duta was evaluated using this model. Combination exposure showed the most potent cell growth inhibitory effect than other ARATs tested in this model.**
3. **Our bone microenvironment model is unique and useful to evaluate the new drug susceptibility testing in prostate cancer cells. This model may help in disclose unknown mechanisms from micro- to clinical bone metastasis in prostate cancer.(Japan patent applying no. C12N 5/09)**

GRANT SUPPORT

This study was supported by Grants-in-Aid for Scientific Research foundation B (KAKENHI: 17H04330) from Japan Society for the Promotion of Science. And Jansen Pharma non-clinical investigator-initiated study.