Effects of genetically engineered human neural stem cells expressing cytosine deaminase and interferon-beta on the growth of lymph node metastatic colorectal adenocarcinoma

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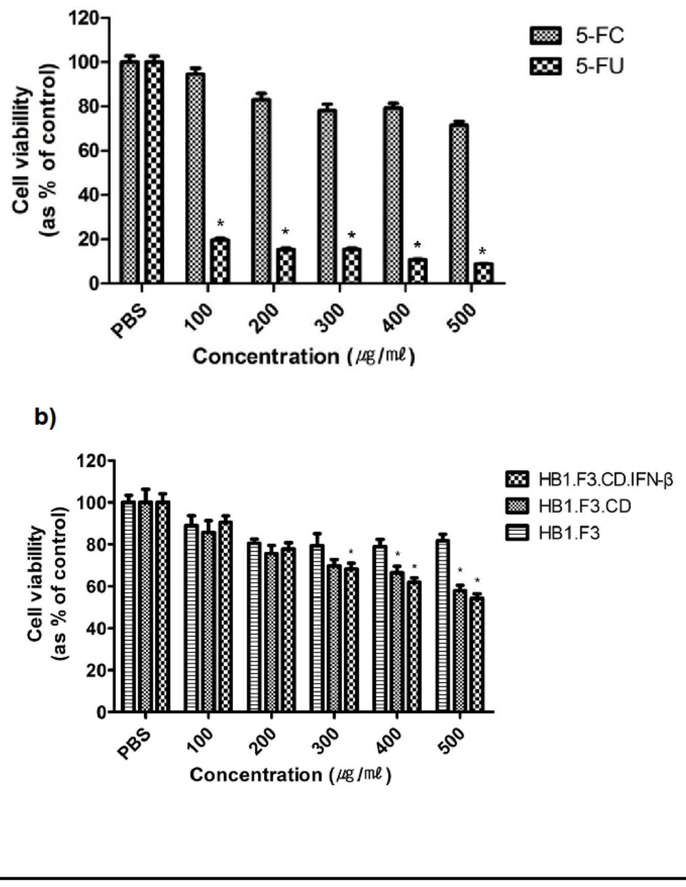
ABSTRACT

Genetically engineered stem cells may be advantageous for gene therapy against various human cancers due to their inherent tumor-tropic properties. In this study, we employed human neural stem cells (HB1.F3; hNSCs) transduced with genes expressing *Escherichia coli* cytosine deaminase (HB1.F3.CD) and human interferon-beta (HB1.F3.CD.IFN-β) as a treatment strategy for human colorectal cancer. CD can convert the prodrug 5-fluorocytosine (5-FC) to its active chemotherapeutic form, 5-fluorouracil (5-FU), which induces a tumor-killing effect through DNA synthesis inhibition. IFN-β also strongly inhibits tumor growth by inducing apoptotic process. In RT-PCR analysis, we confirmed that HB1.F3.CD cells expressed CD gene and HB1.F3.CD.IFN-β cells expressed both CD and IFN-β genes. A modified transwell migration assay showed that HB1.F3.CD and HB1.F3.CD.IFN-β cells selectively migrated toward SW-620 human colorectal cancer cells. When co-cultured with HB1.F3.CD or HB1.F3.CD.IFN-β cells in the presence of 5-FC, the viability of SW-620 cells were significantly reduced. The tumor inhibitory effect was greater with HB1.F3.CD.IFN-β cells, indicating an additional effect of IFN-β to 5-FU. In addition, the tumor-tropic properties of these engineered hNSCs were found to be attributed to chemoattractant molecules secreted by SW-620 cells, SDF-1, c-kit, uPAR, uPA and CCR2. An in vivo assay, hNSC treatment significantly inhibits the growth of colorectal cancer without any virulent effects on the animals. Consequently, the present results represent that engineered hNSCs and prodrug treatment inhibits the growth of human colorectal cancer cells. Therefore, hNSC therapy may be a clinically effective tool for the treatment of human colorectal cancer cancer.

PURPOSE

The present study describes the potential of genetically engineered stem cells (GESTECs) expressing bacterial cytosine deaminase (CD) and human interferon-b (IFN-b) in reducing tumor growth via tumor tropic effect in colorectal cancer animal models. Also, we investigated whether NSCs have a significant migrating capacity for selective targeting via chemoattractant signaling as well as therapeutic effect.

RESULTS



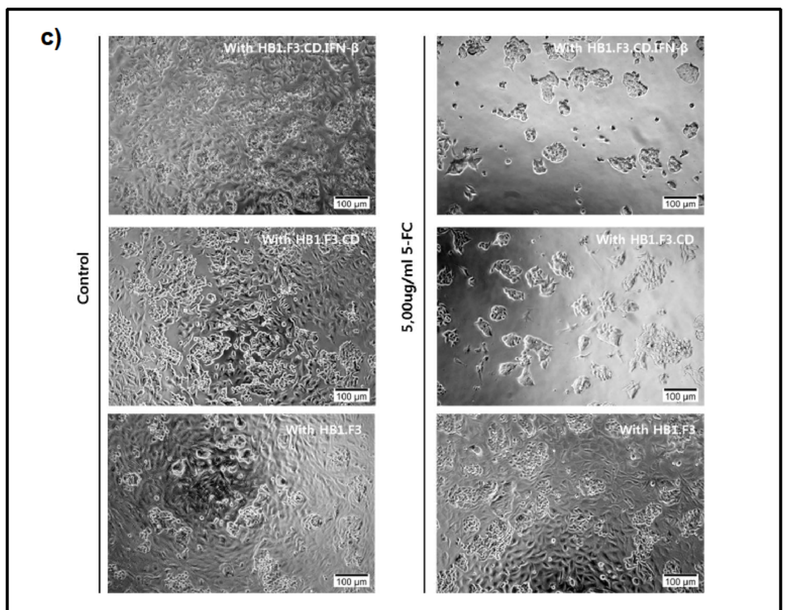
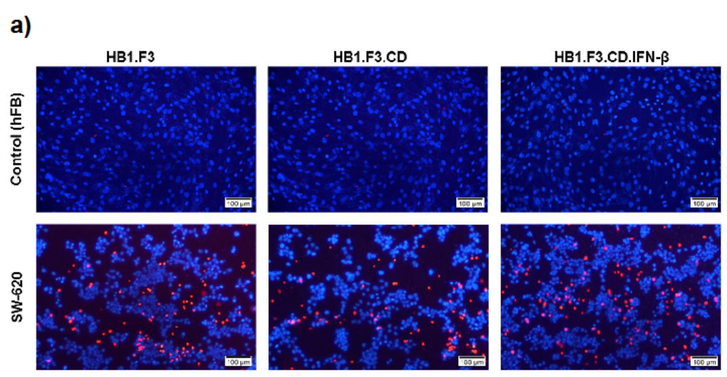


Figure 1. Therapeutic efficacy of HB1.F3.CD and HB1.F3.CD.IFN-β with 5-FC in vitro. a), b) After SW-620 (4×10^3 cells/well) were seeded in 96-well plates for 24 h, stem cells were co-cultured in the presence of 5-FC for another 24 h. After 4 days, the cell viability was measured by MTT assay. A, The cytotoxic effect on colorectal cancer cells of various concentrations of 5-FC or 5-FU (100, 200, 300, 400, and 500 μg/ml) was measured. B, colorectal cancer cells were cultured with HB1.F3, HB1.F3.CD, or HB1.F3.CD.IFN-β hNSCs (8×10^3 cells/well) and treated with increasing concentrations of 5-FC (100, 200, 300, 400, and 500 μg/ml). c) Apoptosis was observed when co-culture with GESTECs through microscope. Images were captured at X20 maginification using an Olympus CKX 41 microscope.



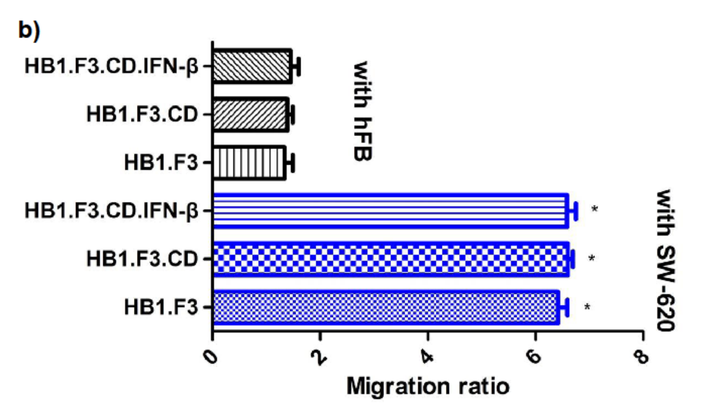
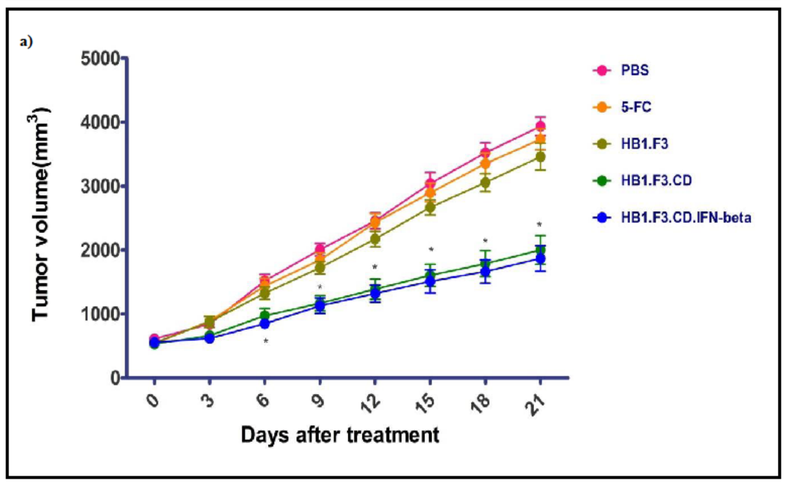


Figure 2. In vitro migration of hNSCs toward Human colorectal cancers (SW-620). (a) Human fibroblast or SW-620 (colorectal cancer cell; $1x10^5$ cells/well) were seeded in the lower wells of 24-well plates. HB1.F3.CD cells ($1x10^5$ cells/well) were stained with CD-Dil and seeded in the fibronectin precoated upper wells of 24-well plates. DAPI staining solution was added to lower wells to observe SW-620 and human fibroblast. Blue stained cells indicated SW-620 or human fibroblast as a control in the lower wells. Red stained cells indicated HB1.F3.CD or HB1.F3.CD.IFN-b cells migrated from the upper wells toward SW-620 or human fibroblast cells. (b) The migration ratio of stem cells was quantitated using image J.



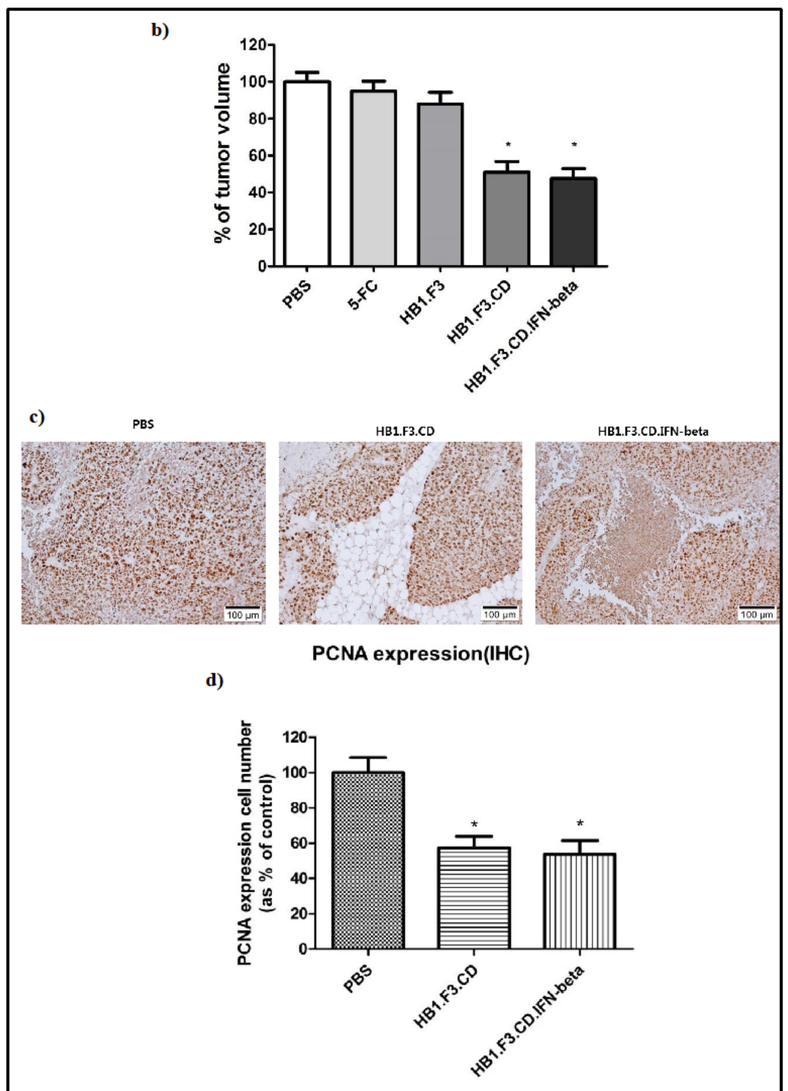


***Figure3.* Xenograft model study and IHC analysis.** a), b) Three week after cancer cell injection, pre-stained hNSCs (1x10^6 cells/mouse) were injected nearby tumor mass. To days late, 5-FC(500mg/kg/day) was treated every day. And the animals were sacrificed 2 days after last injection. Formed tumor size were measured for 3 weeks and the tumor volumes were calculated by 0.5236 x length x width x high. c), d) Histological analysis of tumor mass extracted from mice. PCNA that one of proliferation marker was significantly down-regulated in NSCs treatment group.

CONCLUSION

1. **In vitro co-culture data showed that engineered neural stem cells expressing CD and/or IFN-b not only reduced the viability of SW-620 but also inhibited tumor growth.**
2. **In xenograft cancer model, therapeutic stem cells sufficiently inhibited tumor growth by up to 50%.**
3. **In IHC analysis, proliferation marker was significantly down-regulated in NSCs treatment group.**

REFERENCES

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