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〔ORIGINAL〕

Establishment and characteristics of a cell line derived from osteosarcoma induced by ^{32}P in the rat.

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Abstract

To study the medical treatment of human osteosarcoma there is a need for experimental tumor models Moreover, osteoblastic cell line is a useful model for research of the bone and mineral metabolism. Even today, a few transplantable bone-forming osteosarcomas and their cell lines are available Previously we have reported transplantation of osteosarcoma induced by administration of ^{32}P This tumor retains the osteoid-forming ability even in serial transplantations In the present study, we established the cell line of our transplantable rat osteosarcoma and investigated the characteristics of the cell line The results were as follows

1 A cell line (MSK, Muto-Sato-Kanazawa) of rat osteosarcoma was established
2 The cell line showed intense staining of alkaline phosphatase and collagen synthesis *in vitro.*
3. MSK cell line also maintained the bone-forming ability when it was inoculated subcutaneously into the back of syngenic rats
4. Osteogenesis outside a Millipore filter by MSK cells was demonstrated *in vivo.*
5. It was suggested that MSK cells had not only bone-forming ability but also bone-inductive ability. Therefore, MSK cell line would be a useful model for the study on the property of osteoblast and bone induction

Key words・Cell culture, Osteosarcoma, Osteoblast-like cell, Bone-inductive ability; Cell line

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Introduction

Transplantable osteosarcomas with bone or osteoid forming ability in subsequent transplants are very rare[1-3]

The transplantable osteosarcoma may be a good experimental model for human osteosarcoma and biochemical and morphological investigations on the property of osteoblast There are spontaneous osteosarcoma in mouse (Dunn osteosarcoma[1]), cell lines from rat osteosarcomas (ROS[4]and UMR[5]) and human osteosarcoma (SaOS-2[6]) as representative of available models for its bone-forming ability The bone-forming ability is decreased or lost in most of transplantable osteosarcomas after serial transplantations[7-9] Our previous study reported that a transplantable rat osteosarcoma induced by ^{32}P had a bone-forming ability even in serial transplantation for more than three years[10,11]

Therefore it is expected that the cell line of our osteosarcoma may possess osteoblastic property in serial cultures However, the cell line of our tumor have not yet been established The aim of this study is to establish a cell line of osteosarcoma with bone-forming ability in serial cultures and to investigate the characteristics of the cultured cells

Material and Methods

1) Cell culture

In this study, the material used for cell culture was obtained from 5th generation of rat osteosarcoma induced by administration of ^{32}P which has been maintained in F344 rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan)[11] Pieces of the tumor were removed aseptically and cut into small pieces of about 0 5mm^3 Primary culture was performed by collagenase digestion (0 2% trypsin 37°C) of these tissues The dispersed cells were cultured in α-MEM medium (Wako, Tokyo, Japan) containing 10% fetal bovine serum (Gibco, N Y , U S A), 100 units /ml penicillin and 100μg/ml streptomycin, on a plastic petri dish (Falcon Plastics, Calif , U S A) at 37°C in an atmosphere of 5% CO_2, 95% air The medium was exchanged every 48 hours For subcultures, 5-9 days interval was necessary

2) Light microscopy

The cultured cells before and after the confluent monolayer were fixed in 95% ethanol for 20 minutes and stained either with May-Grunwald Giemsa solution or naphthol AS-BI phosphate (Sigma Chemical Co , St Louis, U S A) as a substrate for alkaline phosphatase activity

3) Electron microscopy

The cultured cells before and after the confluent monolayer were fixed in 2 5% glutaraldehyde in 0 1M phosphate buffer (pH 7 2) for 24 hours The cells were then postfixed in 1% OsO_4 for 30 minutes, dehydrated with graded ethanol and embedded in Epon, using hydroxypropyl methacrylate (HPMA)[12] After polymerization, the thin layer of resin was snapped free from the surface of the culture flask Selected areas were cut out parallel to the surface Sections

were stained wıth uranyl acetate and lead citrate and examined under a JEM-100C transmission electron microscope

4) Establıshment of tumors ın rats from culture cells

Cultured cells at 5 and 13 passages were separated by the use of a polıceman to form a suspension and 0 5ml (apporoximately $1x10^7$ cells) of the suspension was injected subcutaneously ınto the back of syngenic F344 rats to form tumors from the culture cells

5) Bone-ınductive ability of cultured cells

About $1x10^6$ cells were loaded into each dıffusion chambers (Millipore Corporation, Sydney) The pore size of the filters was 0 45μm and they were 150μm thick The chambers were implanted subcutaneously ın the flank of the four F344 rats Eıght weeks after implantation, the anımals were sacrıficed and the chambers with surrounding tissues were removed The

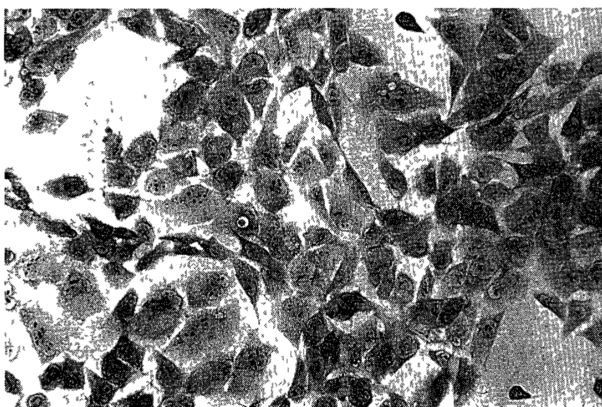


Fıg 1 MSK cells cultured for 3 days The cells are pleomorphıc, varıng between polygonal and fıbroblast-lıke shapes (Gıemsa, x160)

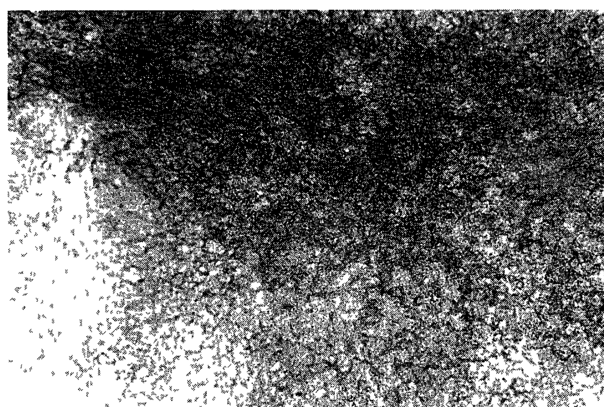


Fıg 2 Alkalıne phosphatase staınıng of the MSK cells Intense alkalıne phosphatase staınıng ıs seen ın the cytoplasm and cell membrane (x80)

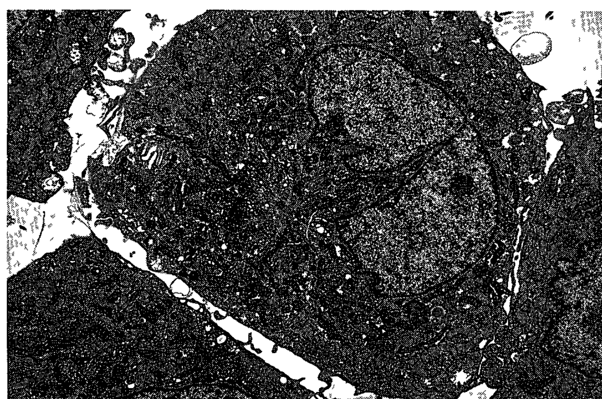


Fıg 3 Electron mıcroscopıc photograph of osteoblast-lıke cells The nucleus ıs ırregular and cytopasm contaıns large numbers of mıtochondrıa and rough endoplasmıc retıculum (x3,500)

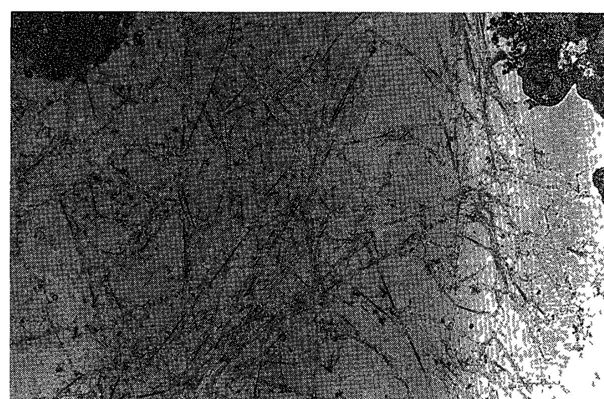


Fıg 4 Electron mıcroscopıc photograph of ıntercellular matrıx Promınent bundles of extracellular fıbrıls are seen (x8,000)

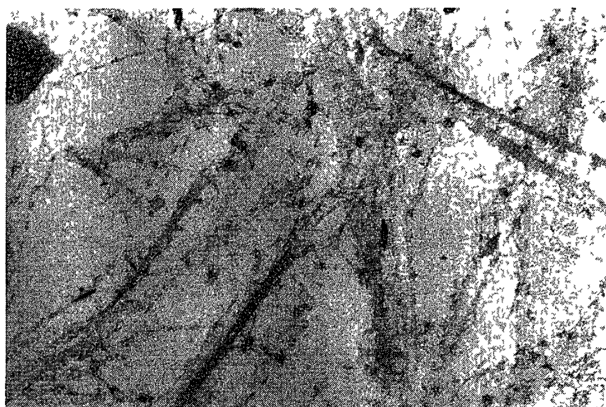


Fig 5 Electron microscopic photograph showing banded collagen fibrils (x35,000)

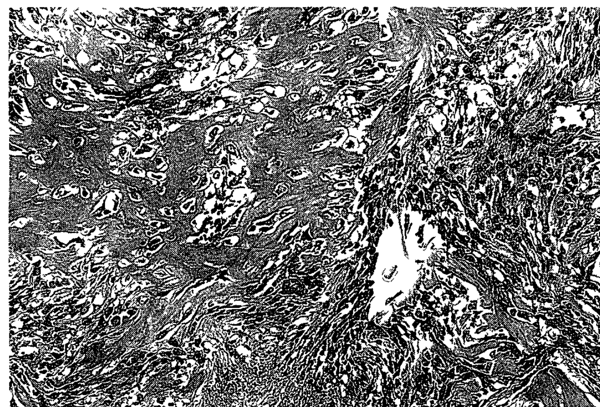


Fig 6 Photomicrograph of tumor on the back of the rat resulted from injection of MSK cells Tumor shows the abundant bone and osteoid tissues (H & E, x40)

specimens were fixed in 10% buffered formalin The disk of tissue was removed from the center of the chamber along with the adjacent filiters with a punch 7mm in diameter and embedded in paraffin Serial cross-sections were cut perpendicular to the filter and stained with hematoxylin and eosin

Results

1) Cell culture and light microscopy

The cultured cells in logarithmic growth phase showed fibroblast-like shape and reached to a confluent monolayer at 7th day and formed island of piled up cells without contact inhibition The shape of the cells were polygonal (Fig 1) The cells cultured for 7 days showed intense staining of alkaline phosphatase (Fig 2)

The cultured cells were successfully maintained for more than one year and passed over 50 subcultures This cell line was named MSK (Muto-Sato-Kanazawa)

2) Electron micorscopy

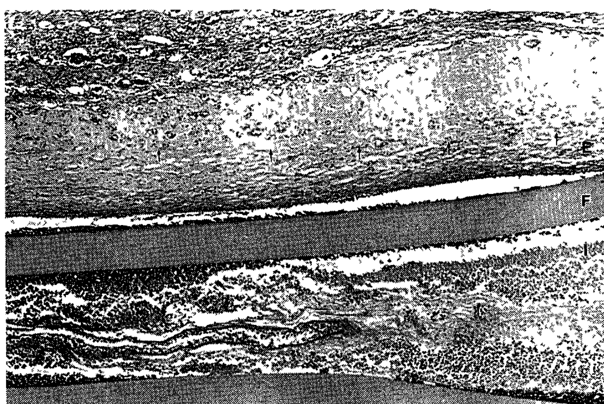
Ultrastructual observation demonstrated a large irregular nucleus, moderate numbers of small mitochondria with high electron density and dilated rough endoplasmic reticulum (Fig 3) In extracellular matrix, prominent bundles of extracellular fibrils were seen (Fig 4) Moreover, typical osmiophilic cross-banding of collagen was also seen (Fig 5)

3) Establishment of tumors from cultured cells

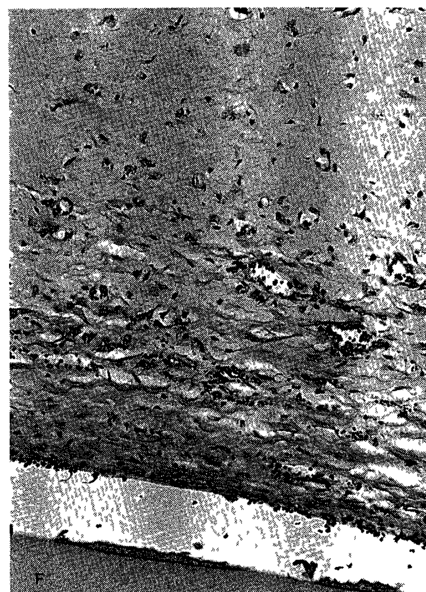
Retransplantation of MSK cells resulted in the development of tumors, showing the osteoid-forming ability that were similar to those of the tissue originally explanted (Fig 6)

4) Bone-inductive ability of MSK cell

In *in vivo* diffusion chamber, osteoid or chondroid-like substances were seen outside the filters of the chambers On the other hand, these substances were not observed at all inside the filters (Fig 7-A, B)



Fgi 7 Photomicrographs of sections inside (I) and outside (E) the filters (F) of the chamber (A) There is a thin layers (arrows) with eosin staining along the external surface of the filter
(H & E, x40)



(B) Under the higher magnification this uniformly stained intercellular substances are seemed like the chondroid tissues (H & E, x200)

Discussion

The purpose of this study was to establish a cell line from osteosarcoma that possesses the osteoblastic property and to investigate the characteristics of the cell lines Rat osteosarcoma was successfully transferred to a cell culture system When osteosarcoma cells were transferred from an *in vivo* environment to a cell culture system, the alternation of cellular properties such as the loss of differentiated properties was usually seen[9] However, our rat osteosarcoma induced by ^{32}P showed the osteoblastic properties such as alkaline phosphatase activity and fibrogenesis of collagen *in vitro*

Moreover, when MSK cells were subcutaneously implanted to the same strain of inbred F344 rats, they formed the same kind of osteosarcoma having bone-forming ability as the original

Osteoblastic cell lines, such as UMR[4], ROS[5], SaOS-2[6] and MC3T3-E1[13] were widely used by now in the study of bone and mineral metabolism MSK cell line established in this study also retained the osteogenetic properties *in vitro* and *in vivo* for more than one year

Among the cell lines derived from osteosarcomas, it has been reported that only a Dunn osteosarcoma secreted a bone inductive substance[6,15,16] Whether the bone-inductive ability exists in MSK cell was also examined in *in vivo* diffusion chamber Mineralization in the diffusion camber was not found However, osteoid or chondroid tissue was noticed outside the filters The shape of the cells in the osteoid tissue differed apparently from that of MSK cells inside the filters On the other hand, any osteoid or chondroid tissue was not seen at all inside the filters From these results, it was suggested that MSK cells had not only bone-forming

abılity but also bone ınductıve abılity Therefore, MSK cell would be a useful model to study bone and mıneral metabolısm The detailed investigation of MSK cells concerning to thıs bone ınductıve abılıty wıll be a subȷect of further study

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抄　録

骨肉腫細胞株はヒト骨肉腫治療の実験モデルになるばかりでなく，骨形成を示すため骨代謝の研究に対しても有用な実験モデルとなりうる。しかし，可移植性骨肉腫株やその細胞株は

今日においても非常に少ない。われわれはすでに^{32}Pで誘発した可移植性骨肉腫を報告した。この腫瘍は継代移植においても骨形成能を維持している。今回の実験はこの骨腫瘍の細胞株を樹立すること，ならびにその生物学的特長を検索することである。

その結果，

1．ラット骨肉腫の細胞株(MSK，武藤一佐藤一金沢）が樹立された。

2．この細胞株は*in vitro*において強いアルカリフォスファターゼ活性とコラーゲン形成能を示した。

3　MSK細胞を同系ラットの背部皮下に移植すると骨形成能を示した。

4　ミリポアフィルターを用いた*in vivo*骨形成実験では，フィルターの外側に骨形成が認められた。

5　以上の結果より，MSK細胞は骨形成能を示すだけでなく骨誘導能の可能性が示唆された。それゆえ，この細胞株は骨芽細胞の特性の検索ばかりでなく骨誘導の研究にも有用なモデルであると考える。