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Adipogenic, osteogenic and myofibrogenic differentiations of a rat malignant fibrous histiocytoma (MFH)-derived cell line, and a relationship of MFH cells with embryonal mesenchymal, perivascular and bone marrow stem cells

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ABSTRACT

Malignant fibrous histiocytoma (MFH) is regarded as an undifferentiated pleomorphic sarcoma with unproven histogenesis. We investigated pathobiological characteristics of a rat MFH cell line (MT-9). Immunocytochemically, MT-9 cells and MT-9-induced tumours reacted to vimentin, A3 (rat MFH cell-specific antibody), macrophage markers and α-SMA (myofibroblastic marker), indicating that MT-9 showed both histiocytic and (myo)fibroblastic features. Adipogenic supplement-added MT-9 showed increased accumulation of lipid droplets. Addition of BMP-2 or osteogenic supplement to MT-9 enhanced osteoblastic markers (ALP activity, osteocalcin mRNA expression and calcification). TGF-β1-treated MT-9 revealed increased numbers of α-SMA-immunopositive cells, and enhanced protein levels of α-SMA and fibronectin, indicating myofibrogenesis. In rat tissues, A3 labelled with immature mesenchymal and perivascular cells in foetuses and neonates, and with marrow stem cells in adults. c-kit mRNA expression was seen in bone marrows and MT-9. Collectively, progenitors of MFH should be sought in lineage of marrow stem cells capable of differentiating into mesenchymal cells.

1. Introduction

Malignant fibrous histiocytoma (MFH) has been reported to be the most common neoplasm of the soft-tissue in the elderly.[1,2] Although MFH is characterised histologically by the presence of histiocytic and fibroblastic cells arranged in a storiform pattern,[1–3] the precise histogenesis and derivation remain undetermined. Besides subcutis, sarcomas with MFH-like histology have been reported to occur in visceral organs and bones,[4,5] and MFH-like histological phenotypes have been seen in parts of fibrosarcomas, leiomyosarcomas, osteosarcomas, malignant schwannomas and liposarcomas of human clinical species.[1,2,6,7] MFH may merely represent a morphological pattern shared by a wide variety of poorly differentiated pleomorphic sarcomas.[2] Based on these observations, we drew a hypothesis that MFH may generate from

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mesenchymal stem cells with multidirectional differentiations.

Knowledge of pathobiological characteristics of cancers is essential to establish effective treatments. Previously, we established a cloned cell line (MT-9) from a spontaneous MFH found in an aged F344 rat.[8,9] The original tumour was demonstrated to consist of an admixure of histiocytic and fibroblastic cells in electron microscopy and enzyme histochemistry.[9] This study was conducted to clarify the differentiation potential of MFH cells which may be induced by stimulating factors already established as suitable inducers. Particularly, we focused on osteogenic, adipogenic and myofibrogenic differentiations of MT-9 cells. Furthermore, using a rat-MFH specific antibody (A3) that we have developed previously,[10] the distribution of A3-positive cells was immunocytochemically investigated in tissues from foetal, neonatal and adult rats, to explore the possible progenitor of MFH cells. This study clearly demonstrated that MFH cells have a capacity for mesenchymal differentiations, and that MFH cells, embryonal mesenchymal cells, perivascular cells and bone marrow stem cells have common antigens recognised by A3. It is likely that the progenitor of MFH cells is involved in the differentiating lineage of marrow stem cells.

2. Materials and methods

2.1. Rat MFH cell line (MT-9)

A parent cell line (MT-P) was prepared from a homotransplantable tumour which had been established from a spontaneous MFH arising in the subcutis of an aged F344 male rat.[8,9] MT-P was cloned twice by the limiting dilution technique, and a cloned cell line (MT-9) was isolated.[8] Eagle's minimum essential medium (E-MEM, Nissui, Tokyo, Japan) containing 10% foetal bovine serum (FBS, Bioserum, UBL, Japan), 0.03% L-glutamine (Nissui), penicillin (100 U/ml) and streptomycin (100 μg/ml) was used as the growth medium. MT-9 cells were cultured in a humidified 5% CO_2 atmosphere at 37 °C as described previously.[8]

2.2. Animals

Pregnant F344 rats and 5-week-old F344 rats were obtained from Charles River Japan (Shiga, Japan). Samples were obtained from foetal rats at gestation days 18 and 21, post-natal rats aged 1, 3, 6, 9, 12 and 15 days, as well as adult rats over 6-weeks-old. These tissue samples and MT-9-induced tumours were fixed in 10% neutral buffered formalin, Zamboni's solution or periodate-lysine-paraformaldehyde (PLP) fixative.[11] The present experiments were conducted in full compliance with our institutional guidelines for animal care.

2.3. Histopathological analyses of MT-9 cells and MT-9-induced tumours

MT-9 cells cultured for 2 days in E-MEM on tissue culture chamber slides (LAB-TEK; Naperville, IL, USA) were fixed in formalin or cold acetone/ethanol (50:50). Formalin-fixed cells were stained with haematoxylin and eosin (HE), and acetone/ethanol-fixed cells were labelled immunocytochemically as described below. For *in vivo* observations, MT-9 cells (10^6/ml) were inoculated subcutaneously into syngeneic rats. Subcutaneous tumours developed were fixed in the above fixatives. Tumour tissue sections were stained with HE, and immunocytochemically labelled.

For immunocytochemical analysis, the avidin–biotin complex (ABC) method (Vectastain ABC Kit; Vector Laboratories Inc., Burlingame, CA, USA) was utilised with the primary antibodies as follows[11]: anti-vimentin (monoclonal; Dako, Corp., Carpinteria, CA, USA; ×200), anti-α-smooth muscle actin (α-SMA; monoclonal; Dako, ×100), ED1 (monoclonal; Chemicon International Inc., Temecula, CA, USA; ×500), ED2 (monoclonal; Serotec Ltd., Tokyo, Japan; ×500), anti-cytokeratin (monoclonal; DAKO, predilution), anti-glial fibrillary acidic protein (GFAP; polyclonal; Dako; × 1000), anti-S-100 protein (polyclonal; Dako; ×200), and the A3 (monoclonal; Trans Genic Inc., Kobe, Japan; ×100). ED1 and ED2 are rat macrophage/histiocyte specific antibodies,[11–14] and A3 antibody is highly specific for rat MFH cells.[10] Formalin-fixed deparaffinised sections were used for vimentin, cytokeratin, α-SMA, ED1, S-100 protein and GFAP.[11] Zamboni's solution-fixed deparaffinised sections and PLP-fixed deparaffinised sections were used for ED2 and A3, respectively.

The following procedures were applied in common to tissue sections and culture cell sections. The sections were incubated with 0.5–5% H_2O_2 for 10 min to quench endogenous peroxidase. The slides were then treated with 5% non-fat milk for 45 min, followed by incubation with each primary antibody for 14 h at 4 °C. Next, 30 min of incubation with biotinylated goat anti-mouse antibody for monoclonal antibodies or with goat anti-rabbit antibody for polyclonal antibodies was performed. Final incubation was carried out for 30 min with an avidin-biotinylated peroxidase complex, and positive reactions were visualised with 3,3′-diaminobenzidine (DAB). Non-immunised mouse or rabbit serum was used for negative controls.

2.4. Adipogenic differentiation

We used two different adipogenic inducers. The 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15-dPGJ_2) was obtained from BIOMOL International (PA, USA),[15] and adipogenic supplement (including insulin, 3-isobutyl-1-methylxanthin, indomethacin and dexamethasone (DMSO)), which was developed as an adipogenic stimulus for bone marrow stem cells,[16] was purchased from Dainippon Sumitomo Pharma Co. Ltd. (Esaka, Japan). For the experiment of 15-dPGJ_2, cells (0.5×10^4/ml) were incubated in E-MEM containing 15-dPGJ_2(5μM) (diluted in DMSO) or an equivalent DMSO (vehicle control). The E-MEM containing 15-dPGJ_2 or vehicle was changed every 2 days. After addition, cells were fixed in formalin at 4, 8 or 25 days, and stained with Oil-Red-O. For adipogenic supplement, cells (0.5×10^4/ml) were incubated in E-MEM containing the supplement (0 or 50 μl/ml);[16] after addition, cells were fixed in formalin at 4, 8 and 12 days, and stained with Oil-Red-O. The number of colonies of cells with Oil-Red-O-positive lipid droplets was counted in four different fields at 200×, and the ratio of lipid droplets per cytoplasm was evaluated in 10 randomly-se-