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Copper-64-diacetyl-bis (N^4-methylthiosemicarbazone) accumulates in rich regions of $CD133^+$ highly tumorigenic cells in mouse colon carcinoma☆

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Abstract

Introduction: ^{64}Cu-diacetyl-bis (N^4-methylthiosemicarbazone) (^{64}Cu-ATSM) is a potential imaging agent of hypoxic tumor for use with PET. Recent literature demonstrated that cancer cells expressing CD133, which is a frequently used marker for so-called cancer stem cells or cancer stem cell-like cells (collectively referred to here as CSCs), contribute to tumor's therapeutic resistance and metastasis ability. Culturing under hypoxia is also reported to enlarge the proportion of $CD133^+$ cells, which would indicate survival advantage of $CD133^+$ cells under hypoxia. Here, we investigated the relationships between ^{64}Cu-ATSM accumulation and existence of $CD133^+$ cells using mouse colon carcinoma (colon-26) tumor.

Methods: Intratumor distribution of ^{64}Cu-ATSM and ^{18}F-fluorodeoxyglucose (^{18}FDG) was compared with immunohistochemical staining for CD133 with a colon-26 model. In vitro characterization of $CD133^+$ colon-26 cells was also performed.

Results: In colon-26 tumors, ^{64}Cu-ATSM localized preferentially in regions with a high density of $CD133^+$ cells. The percentage of $CD133^+$ cells was 11-fold higher in ^{64}Cu-ATSM high-uptake regions compared with ^{18}FDG high- (but ^{64}Cu-ATSM low-) uptake regions. $CD133^+$ colon-26 cells showed characteristics previously linked with CSCs in other cancer cell lines, such as high colony-forming ability, high tumor-initiating ability and enrichment under hypoxic cultivation. The proportion of $CD133^+$ cells was enlarged by culturing under glucose starvation as well as hypoxia, and ^{64}Cu-ATSM uptake was increased under such conditions.

Conclusions: Our findings showed that, in colon-26 tumors, ^{64}Cu-ATSM accumulates in rich regions of $CD133^+$ cells with characteristics of CSCs. Therefore ^{64}Cu-ATSM could be a potential imaging agent for rich regions of $CD133^+$ cells, associated with CSCs, within tumors.

Keywords: Highly tumorigenic cell; Cancer stem cell; CD133; Colon carcinoma; ^{64}Cu-ATSM

1. Introduction

Hypoxia frequently occurs in tumors due to poor vascularization and often tight packing of cancer cells, and it is considered to exacerbate malignancy: it is associated with adverse prognosis due to failures in radiotherapy and chemotherapy and to tumor metastasis [1]. Accordingly, it is of significant importance to identify and characterize hypoxic tumors.

Noninvasive methods for detection of hypoxic tumor have been intensively developed [2]. Of these methods,

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radiolabeled Cu-diacetyl-bis (N^4-methylthiosemicarbazone) (Cu-ATSM) is a promising imaging agent for PET [3–10]. To date, Cu-ATSM is known to accumulate in hypoxic environments in many kinds of tumor cells in vitro [6,11,12]. Clinical studies have demonstrated that hypoxic tumors assessed by Cu-ATSM uptake are associated with the tumors' therapeutic resistance and metastasis ability [3,13–15]. The mechanism of Cu-ATSM accumulation in hypoxic regions has been reported [5,11,16–18]: under highly reduced intracellular conditions such as hypoxia, Cu(II) in Cu-ATSM is reduced to Cu(I), instantly released from the ATSM ligand and trapped in the cells [5,11,16,17]. It is also reported that distribution of Cu-ATSM in tumors differs from that of ^{18}F-fluorodeoxyglucose (^{18}FDG), a commonly used PET imaging tracer of glucose uptake in tumor cells [9,19]. Tanaka et al. [9] reported that Cu-ATSM has its highest uptake in regions that are hypovascular, undergoing cell cycle arrest but little necrosis, while ^{18}FDG targets regions of hypervascularity and cell proliferation going to necrosis.

Hypothesis of "cancer stem cell" has been recently evolving. According to the accumulating data, the existence of a certain type of cancer cells called cancer stem cells or cancer stem cell-like cells, which are collectively referred to here as CSCs, contributes to therapeutic resistance and ability to metastasize [20,21]. CSCs are recognized to possess resistance to apoptosis triggered by extracellular stressors like radiotherapy and chemotherapy, largely because many are resting or engaged in DNA damage repair [21–23]. In addition, CSCs demonstrate high expression of anti-apoptotic proteins (e.g., the Bcl-2 family) and ATP-binding cassette transporter proteins that may contribute to resistance to apoptosis caused by extracellular stress [21]. It is also shown that in vitro culturing of cancer cells under hypoxia increased the proportion of CSCs compared with cells cultured under normoxia, which indicates that CSCs would have survival advantage under hypoxic conditions [24–27]. CSCs therefore share many characteristics with regions that accumulate Cu-ATSM. Both seem to (1) survive under hypoxia, (2) be in a resting state, (3) demonstrate therapeutic resistance and (4) possess high metastatic ability.

CD133 (prominin-1) is a frequently used marker to identify the CSCs in various human and murine cancers, such as brain, prostate, breast, liver and colon [28–35]. It has been demonstrated that the CD133$^+$ cancer cells show high colony-forming and tumor-initiating ability and enrichment under hypoxic cultivation [20,21,24–38]. The CD133$^+$ cells are also recognized to contribute to therapeutic resistance and metastatic ability of tumors [22,23]. Consequently, we hypothesized that hypoxic tumor regions visualized by Cu-ATSM would contain higher proportions of CD133$^+$ cells, which are considered to be candidates of CSCs.

In this study, we compared intratumoral distribution of ^{64}Cu-ATSM and ^{18}FDG to the distribution of cancer cells expressing CD133 in a mouse colon cancer (colon-26) tumor model, in which distribution of ^{64}Cu-ATSM and ^{18}FDG and intratumoral phenotype (such as blood vessel density, cell proliferation and type of cell death) have been described in a previous study [9]. We also performed characterization of CD133$^+$ colon-26 cancer cells and examined ^{64}Cu-ATSM uptake in colon-26 cells: among them, we confirmed that CD133$^+$ colon-26 cells possessed characteristics previously linked with CSCs in other cancer cell lines, such as high colony-forming ability, high tumor-initiating ability and enrichment under hypoxic cultivation.

2. Materials and methods

2.1. Cell lines and growth

A mouse colon carcinoma cell line, colon-26 (TKG 0518; Cell Resource Centre for Biomedical Research, Tohoku University, Sendai, Japan), was used in this study. The cells were incubated in a humidified atmosphere of 5% CO_2 in air at 37°C. Dulbecco's Modified Eagle's Medium (DMEM 11995-065; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum and antibiotics was used for cell growth. Exponentially growing cells were used for the study. The cells were trypsinized to detach them from the plates and were counted by the trypan blue dye-exclusion method for assays.

2.2. Radioactive tracers

For ^{64}Cu production, gold disc (25 mm diameter×2 mm deep with a dimple in the middle) plated with enriched ^{64}Ni (98.1%, Trace Science International, Ontario, Canada) by electrodeposition was prepared according to a method previously reported [10,39]. The gold discs were irradiated by 11-MeV protons with an RDS Eclipse biomedical cyclotron (Siemens, Göteborg, Sweden). Separation of ^{64}Cu and synthesis of ^{64}Cu-ATSM were performed based on a previously reported procedure [10]. The radiochemical purity of the resultant ^{64}Cu-ATSM was >99%, which was determined by HPLC (LC-10ADVP; Shimadzu, Kyoto, Japan) with a reversed-phase column (4.6×150 mm 5C18-AR-II, Waters, Milford, MA, USA). Radioactivity was measured by a radio analyzer (RLC-700, Aloka, Tokyo, Japan). Elution solvent was CH_3CN/water (75:25, vol/vol) at a flow rate of 1.0 ml/min. ^{18}FDG was synthesized with an automated ^{18}FDG synthesizing system (JFE, Tokyo, Japan) as previously described [9,40]. The specific activity of ^{64}Cu-ATSM was 56 GBq/μmol and that of ^{18}FDG was 20 to 50 GBq/μmol.

2.3. Animals

All animal experiments and procedures were conducted in compliance with the Animal Treatment Regulations of the University of Fukui, Japan. BALB/c male mice (6 weeks of age, 20 to 25 g of body weight) were obtained from Japan SLC (Shidzuoka, Japan). Before the experiments, the mice were kept undisturbed for at least 1 week.