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Biology Contribution

Relationship Between Chromatin Structure and Sensitivity to Molecularly Targeted Auger Electron Radiation Therapy

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Summary

The effect of chromatin structure on the cytotoxicity of radiopharmaceuticals is largely unknown. The evidence presented here suggests that the relaxation of chromatin, through exposure to HDAC inhibitors for example, enhances the cytotoxic effect of ^{111}In-DTPA-hEGF, an Auger electron-emitting anticancer radiopharmaceutical. In contrast NaCl-induced chromatin condensation resulted in a radioprotective outcome. These results indicate that the current interest in combining external radiation with HDAC inhibitors is also

Purpose: The open structure of euchromatin renders it susceptible to DNA damage by ionizing radiation (IR) compared with compact heterochromatin. The effect of chromatin configuration on the efficacy of Auger electron radiotherapy was investigated.

Methods and Materials: Chromatin structure was altered in MDA-MB-468 and 231-H2N human breast cancer cells by suberoylanilide hydroxamic acid (SAHA), 5-aza-2-deoxycytidine, or hypertonic treatment. The extent and duration of chromatin structural changes were evaluated using the micrococcal nuclease assay. DNA damage (γH2AX assay) and clonogenic survival were evaluated after exposure to ^{111}In-DTPA-hEGF, an Auger electron-emitting radiopharmaceutical, or IR. The intracellular distribution of ^{111}In-DTPA-hEGF after chromatin modification was investigated in cell fractionation experiments.

Results: Chromatin remained condensed for up to 20 minutes after NaCl and in a relaxed state 24 hours after SAHA treatment. The number of γH2AX foci per cell was greater in MDA-MB-468 and 231-H2N cells after IR (0.5 Gy) plus SAHA (1 μM) compared with IR alone (16 ± 0.6 and 14 ± 0.3 vs. 12 ± 0.4 and 11 ± 0.2, respectively). More γH2AX foci were observed in MDA-MB-468 and 231-H2N cells exposed to ^{111}In-DTPA-hEGF (6 MBq/μg) plus SAHA vs. ^{111}In-DTPA-hEGF alone (11 ± 0.3 and 12 ± 0.7 vs. 9 ± 0.4 and 7 ± 0.3, respectively). 5-aza-2-deoxycytidine enhanced the DNA damage caused by IR and ^{111}In-DTPA-hEGF. Clonogenic survival was reduced in MDA-MB-468 and 231-H2N cells after IR (6 Gy) plus SAHA (1 μM) vs. IR alone (0.6% ± 0.01 and 0.3% ± 0.2 vs. 5.8% ± 0.2 and 2% ± 0.1, respectively) and after ^{111}In-DTPA-hEGF plus SAHA compared to ^{111}In-DTPA-hEGF alone (21% ± 0.4% and 19% ± 4.6 vs. 33% ± 2.3 and 32% ± 3.7). SAHA did not affect ^{111}In-DTPA-hEGF nuclear localization. Hypertonic treatment resulted in fewer γH2AX foci per cell after IR and ^{111}In-DTPA-hEGF compared to controls but did not significantly alter clonogenic survival.

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relevant to molecularly targeted radiation therapy.

Conclusions: Chromatin structure affects DNA damage and cell survival after exposure to Auger electron radiation. © 2012 Elsevier Inc.

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Introduction

Chromatin structure influences radiosensitivity when the extent of DNA damage in compact, transcriptionally inactive heterochromatin is compared with that in loose, transcriptionally active euchromatin after ionizing radiation (IR) (1–3). In euchromatin, which can be increased through histone depletion, the amount of DNA damage increases after IR compared with nonhistone depleted controls (4). By contrast, reduction in euchromatin caused by three-dimensional cell growth results in radioprotection compared with cells grown as a monolayer (5).

Interest in epigenetics has led to the development of drugs that modify the acetylation of histones or the methylation of CpG islands in gene promoters, leading to disrupted gene transcription and cell cycle progression (6, 7). Vorinostat (suberoylanilide hydroxamic acid, SAHA) inhibits histone deacetylases (HDAC), resulting in excessive acetylation of histones. 5-aza-2-deoxycytidine, which inhibits DNA methyltransferase, causes hypomethylation of CpG islands, resulting in chromatin relaxation and reexpression of silenced genes. Both drugs are used clinically: SAHA for cutaneous lymphoma and 5-aza-2-deoxycytidine for myelodysplastic syndrome (8). Several HDAC inhibitors sensitize cells to IR (9).

Although the impact of chromatin structure on cell sensitivity to IR has been studied extensively, little is known about its effect on DNA damage and cell killing caused by anticancer radiopharmaceuticals. ^{111}Indium-labeled human epidermal growth factor (^{111}In-DTPA-hEGF) is an Auger electron–emitting agent that targets epidermal growth factor (EGF) receptor (EGFR)-overexpressing cells (10). ^{111}In-DTPA-hEGF binds the EGFR, internalizes, translocates to the nucleus, and associates with chromatin. Nuclear localization of ^{111}In-DTPA-hEGF is required for cytotoxicity because ^{111}In releases short-range Auger electrons as it decays (11). We hypothesized that chromatin configuration could affect the accessibility of DNA to intranuclear radionuclides and so may be an important determinant of the cytotoxicity of Auger electron radiotherapy.

Methods and Materials

Cell culture

Human breast cancer cell lines MDA-MB-468 and 231-H2N were obtained from American Type Culture Collection and Robert Kerbel, Sunnybrook Health Sciences Centre, Toronto, Canada, respectively. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS; Invitrogen, Paisley, UK), 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C in 5% CO_2. Hypertonic medium was prepared by adding NaCl to FBS-containing medium (0.15 M NaCl) to a final concentration of 0.5 M. Reagents were from Sigma-Aldrich, Dorset, UK, unless stated otherwise.

Radiopharmaceutical

^{111}In chloride (PerkinElmer, Waltham, MA) was used to synthesize ^{111}In-diethylene triamine pentaacetic acid (DTPA)-hEGF using a kit provided by Raymond Reilly, University of Toronto, Canada. Specific activity was 6 to 67 MBq/μg hEGF. Radiochemical purity was 50–90%.

Drugs

SAHA (Cayman Chemical, Ann Arbor, MI) and 5-aza-2-deoxycytidine were dissolved in dimethyl sulfoxide (DMSO) at 1.5 mM and in phosphate-buffered saline (PBS) at 4.38 μM, respectively; sterilized through a Millex GV 0.22-μM filter (Millipore, MA); and stored at −80°C. The maximum concentration of DMSO in cells was 0.066%.

Western blot analysis

Cells were cultured with or without SAHA (1 μM, 24 hours) and resuspended in lysis buffer (2% SDS, 100 mM dithiothreitol, 10% glycerol, and 60 mM Tris pH 6.8). The bicinchoninic acid protein assay (Pierce, Thermo Scientific, IL) was used to enable equal protein loading. Gel electrophoresis was performed, followed by Western blotting. Membranes were blocked in 5% milk/PBS/Tween (0.1%) and probed with anti-H3Ac (Active Motif, Rixensart, Belgium) or EGFR (Santa Cruz Biotechnology, CA) antibodies. β-actin and H3 antibodies (both Abcam, Cambridge, MA) were used as loading controls. Secondary horseradish peroxidase-conjugated antibodies (Promega, Southampton, UK, and Santa Cruz Biotechnology, CA) were used to detect proteins by enhanced chemiluminescence (Pierce, Thermo Scientific). Results were quantified by densitometry (Image J, National Institutes of Health, USA), and fold induction was normalized to control samples.

Micrococcal nuclease assay

231-H2N cells were cultured with or without SAHA (1 μM, 24 hours) or DMSO or 0.5 M NaCl (30 minutes) and washed in fresh isotonic medium, and nuclei were isolated from 1×10^7 cells either immediately or at selected times after washoff. Micrococcal nuclease (MNase) digestion of chromatin was performed as described (12). Briefly, nuclei were digested at 25°C with 0.5U MNase in 1 mM $CaCl_2$-containing digestion buffer. Digestion was terminated with ethylenediaminetetraacetic acid (pH 8.0) every minute for 5 minutes, and proteinase K and SDS were then added. DNA was purified, separated alongside a 100-bp marker (New England Biolabs, Hitchin, UK) by electrophoresis in 1.2% agarose gel containing ethidium bromide, and visualized by