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Remodeling epigenetic modifications at tumor suppressor gene promoters with bovine oocyte extract

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Abstract

Background aims. Epigenetic silencing of tumor suppressor genes by aberrant DNA methylation and histone modifications at their promoter regions plays an important role in the initiation and progression of cancer. The therapeutic effect of the widely used epigenetic drugs, including DNA methyltransferase inhibitors and histone deacetylase inhibitors, remains unsatisfactory. One important underlying factor in the ineffectiveness of these drugs is that their actions lack specificity. *Methods.* To investigate whether oocyte extract can be used for epigenetic re-programming of cancer cells, H460 human lung cancer cells were reversibly permeabilized and incubated with bovine oocyte extract. *Results.* Bisulfite sequencing showed that bovine oocyte extract induced significant demethylation at hypermethylated promoter CpG islands of the tumor suppressor genes *RUNX3* and *CDH1*; however, the DNA methylation levels of repetitive sequences were not affected. Chromatin immunoprecipitation showed that bovine oocyte extract significantly reduced transcriptionally repressive histone modifications and increased transcriptionally activating histone modifications at the promoter regions of *RUNX3* and *CDH1*. Bovine oocyte extract reactivated the expression of *RUNX3* and *CDH1* at both the messenger RNA and the protein levels without up-regulating the transcription of pluripotency-associated genes. At the functional level, anchorage-independent proliferation, migration and invasion of H460 cells was strongly inhibited. *Conclusions.* These results demonstrate that bovine oocyte extract reactivates epigenetically silenced tumor suppressor genes by remodeling the epigenetic modifications at their promoter regions. Bovine oocyte extract may provide a useful tool for investigating epigenetic mechanisms in cancer and a valuable source for developing novel safe therapeutic approaches that target epigenetic alterations.

Key Words: *bovine oocyte extract, epigenetic modification, remodeling, tumor suppressor gene*

Introduction

During the initiation and progression of cancer, tumor suppressor gene (TSG) promoter regions accumulate numerous epigenetic abnormalities, including hypermethylation of CpG islands, increase in transcriptionally repressive histone modifications and decrease in transcriptionally activating histone modifications (1–3). These aberrant epigenetic modifications induce a compact chromatin structure at TSG promoter regions that prevents DNA access by transcription factors, resulting in the silencing of many key TSGs (1–3). In contrast to genetic changes, epigenetic modifications are reversible. Reactivating TSG expression by remodeling the epigenetic modifications at their promoter regions represents a promising strategy for reversal of malignancy.

At the present time, DNA methyltransferase inhibitors and histone deacetylase inhibitors are widely used to reverse DNA hypermethylation and increase histone acetylation modifications at TSG promoters in cancer cells. However, these drugs work by general inhibition of the corresponding epigenetic enzymes rather than by direct action on chromatin regions with abnormal structures (1–3). This mechanism of action leads to a loss of specificity. In addition to changing the epigenetic modifications at TSG promoter regions, these drugs induce hypomethylation of repetitive sequences and activate the transcription of oncogenic pluripotency-associated genes (4–7). For example, treatment of rat chondrosarcoma, human lung adenocarcinoma and human acute lymphoblastic leukemia cells with 5-aza-2-deoxycytidine leads to hypomethylation of long interspersed repetitive element and satellite DNA sequences (4–6). Treatment of human prostate cancer cells with histone deacetylase inhibitor valproic acid up-regulates the expression of *SOX2* and *NANOG* and induces cancer

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stem-like cell characteristics (7). The non-specific effects of these drugs pose a risk for their clinical application (4,7). It is necessary to develop new, more specific approaches to reverse the epigenetic abnormalities at TSG promoters in cancer cells.

The re-programming effect of ooplasm during the development of fertilized oocytes and nuclear transfer embryos has been extensively investigated. The large number of epigenetic re-programming factors stored in ooplasm can efficiently remodel the epigenetic modifications of sperm and donor cell chromatin (8,9). The activity of these epigenetic re-programming factors is retained in oocyte extract and can be used to remodel the epigenetic modifications of normal somatic cells. Bian *et al.* (10) incubated reversibly permeabilized mouse fibroblasts in axolotl oocyte extract; the molecules present in axolotl oocyte extract significantly decreased the H3K9me3 and DNA methylation levels and increased the H3K9Ac level of the somatic chromatin. Similarly, Ganier *et al.* (11) induced significant reduction in H3K9me3, H3K9me2 and H3K27me3 levels of somatic nuclei by incubating reversibly permeabilized mouse fibroblasts in *Xenopus* oocyte extract. Treatment of reversibly permeabilized fibroblasts with porcine oocyte extract by Miyamoto *et al.* (12) also led to increased H3K9Ac levels in somatic nuclei. The oocyte extract-induced epigenetic remodeling is mediated by soluble re-programming factors, which travel into permeabilized cells during incubation and act directly on the epigenetic modifications in specific chromatin regions (10–13). This system provides a useful avenue for re-programming cell fate. However, the existing studies have focused on re-programming normal somatic cells; no studies have investigated whether the re-programming capability of oocyte extracts can be used to reverse the epigenetic abnormalities at TSG promoter regions in cancer cells.

Using H460 human lung cancer cells and bovine oocyte extract, we performed the first study to determine whether cancer cells can be re-programmed by incubation with oocyte extract. Treatment specifically reversed CpG island hypermethylation and remodeled histone modification patterns within TSG promoter regions, leading to the reactivation of the expression of epigenetically silenced TSGs. Also, treatment with oocyte extract strongly inhibited the malignant cancer cell phenotype, suggesting possible therapeutic applications for bovine oocyte extracts.

Methods

Bovine oocyte collection

Ovaries were collected from freshly slaughtered healthy cows and transported to the laboratory within 3 h. Cumulus oocyte complexes were aspirated from follicles 2–8 mm in diameter. Complexes with compact cumulus cells were collected, washed three times in tissue culture medium-199 and repeatedly pipetted to remove cumulus cells. The released oocytes with even cytoplasm were digested with 0.5% pronase for 5 min to remove the zona pellucida.

Bovine oocyte extract preparation

Bovine oocyte extract was prepared as described previously with minor changes (12). Briefly, the zona-free oocytes were transferred into 1.5-mL tubes and washed three times with extraction buffer (5 mmol/L $MgCl_2$, 60 mmol/L NaCl, 2 mmol/L β-mercaptoethanol, protease inhibitor cocktail [Sigma-Aldrich, St Louis, MO, USA], 5 mmol/L ethylene glycol tetraacetic acid and 50 mmol/L *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid, pH 7.4) containing an energy-regenerating system (2 mmol/L adenosine triphosphate, 20 mmol/L phosphocreatine, 20 U/mL creatine kinase and 2 mmol/L guanosine triphosphate). The washed oocytes were re-suspended (100 oocytes/μL) in extraction buffer containing the energy-regenerating system and disrupted by centrifugation twice at 20,800*g* for 30 min at 4°C. The lysate was mixed by pipetting on ice and centrifuged at 5000*g* for 10 min at 4°C, and the supernatant was harvested as the oocyte extract.

Oocyte extract treatment

The human non-small cell lung cancer cell line NCI-H460 was cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) medium supplemented with 10% fetal bovine serum at 37°C at 5% CO_2. To permeabilize the cells, 2×10^6 H460 cells were suspendedin 20 μg/mL digitonin solution for 2 min. Permeabilization was assessed by monitoring the uptake of 70 kDa fluorescein isothiocyanate-dextran (40 μg/mL) in a separate aliquot after re-sealing plasma membranes (14). The permeabilization efficiency under our conditions was >80%. The permeabilized cells were suspended in bovine oocyte extract (5000 cells/10 μL extract; extract-treated cells) or an equal volume of extraction buffer (buffer-treated cells) at 38.5°C for 3.5 h with occasional tapping. For membrane re-sealing, the cell suspension was diluted with 1 mL RPMI-1640 containing 2 mmol/L $CaCl_2$ and incubated for 2 h at 37°C. After pelleting by centrifugation at 400 *g* for 5 min, the cells were subjected to normal culture or used directly for assays.

Bisulfite sequencing

DNA was isolated from H460 cells using the Wizard SV Genomic DNA Purification System (Promega, Southampton, UK). Bisulfite conversion