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Effects of Irradiation on the Expression of Surface Antigens in Human Ovarian Cancer[1]

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Tumor cells from four established human ovarian carcinoma cell lines were analyzed for their expression of surface antigens including MHC Class I, Class II, ICAM-1, and the tumor-associated antigens CA 125 and Her2-neu before and after exposure to high doses of gamma irradiation. All four ovarian cell lines expressed variable levels of MHC Class I and Her2-neu. ICAM-1 antigens were expressed in only two cell lines and Class II and CA 125 surface antigens were absent in all the cell lines evaluated. Exposure to high doses of gamma irradiation (i.e., 5000 to 10,000 cGy) significantly and consistently increased the expression of all surface antigens present on the cells prior to irradiation. Importantly, the irradiation-induced upregulation was persistent and lasted until all the cells died. Irradiation was unable to induce neoexpression of antigens previously not expressed by the cells (i.e., MHC Class II or ICAM-1). These findings may partially explain the increased immunogenicity of tumor cells following irradiation and may suggest enhanced immune recognition in tumor tissue in patients receiving radiation therapy.

INTRODUCTION

Cell surface antigens are important for both recognition and destruction of tumor cells by the host immune system. For a specific immune response to ensue, T cells must recognize a target peptide in the context of a major histocompatibility complex (MHC)[3] molecule. However, additional costimulatory molecules such as ICAM-1 are also needed when the avidity between the T cell receptor an its target antigen on the cell surface is weak [1]. Levels of expression of these cell surface antigens are important in determining whether tumor cells are immunogenic [2, 3] and therefore methods which upregulate their expression may facilitate more efficient host–tumor interactions. In this regard, several studies have described an increase in antigen expression after exposure of tumor cells to certain cytokines, particularly IFN(s) and TNF-α [4, 5], and such tumor cell have been shown to be more immunogenic as determined by *in vitro* cytotoxicity assays [4–6]. Moreover, *in vivo* studies have shown that weakly immunogenic murine tumors could become much more immunogenic following exposure to high–dose gamma irradiation [7, 8]. However, few studies have described the effects of high doses of irradiation on the expression of cell surface antigens on tumor cells, and no studies have been published evaluating such effects on human epithelial ovarian cancer cells.

The physiologic and cellular changes associated with high doses of irradiation have been well documented [for review, see 9]. However, it has only recently been shown that after irradiation an increased synthesis of specific proteins, including MHC Class I antigens, has been observed. Hauser *et al.* [10] reported an increased expression of MHC Class I molecules after fractionated doses of irradiation on a murine melanoma cell line *in vitro* and Hareyama *et al.* [11] demonstrated an increased expression of MHC Class I and carcinoembryonic antigen after lethal irradiation of a human gastric adenocarcinoma cell line.

Such findings have become more important in the past few years due to advances in genetics, immunology, and molecular biology that have provided high efficiency gene transfer systems for vaccine approaches for the treatment of cancer. The success in the use of genetically altered tumor cells (GATC) secreting immuno-enhancing cytokines to

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[3] Abbreviations used: PPC, percentage of positive cells; MFI, mean channel fluorescence intensity; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ MHC, major histocompatibility complex; ICAM-1, intercellular adhesion molecule-1.

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TABLE 1

Expression of Surface Antigens on Four Established Ovarian Cell Lines

Cell line		HLA Class I	HLA Class II	ICAM-1	CA 125	Her-2/neu
UCI-101	PPC[a]	92.2 ± 8.6	ND	87.0 ± 12.3	ND	95.5 ± 5.0
	MFI[b]	58.5 ± 17.5		92.2 ± 48.2		30.7 ± 14.4
UCI-107	PPC[a]	98.3 ± 1.6	ND	ND	ND	95.7 ± 2.5
	MFI[b]	330.6 ± 109.6				48.0 ± 33.6
SKOV-3	PPC[a]	100.0 ± 0.0	ND	ND	ND	100.0 ± 0.0
	MFI[b]	834.3 ± 334.6				224.3 ± 14.4
T-222	PPC[a]	97.2 ± 3.4	ND	99.6 ± 0.5	ND	85.5 ± 25.6
	MFI[b]	41.8 ± 20.3		43.8 ± 18.0		21.5 ± 10.9

Note. Membrane expression of MHC proteins and TAA in established cell lines was examined by FACS analysis. ND, not detectable.

[a] PPC, percentage of positive cells (values are expressed as means ± standard deviation).

[b] MFI, mean channel fluorescence intensity (values are expressed as means ± standard deviation).

stimulate systemic immunity against parental tumor cells in many murine models has stimulated clinical investigators to design human trials in an attempt to translate such results for human cancer immunotherapy. However, allogeneic or autologous human tumor vaccines require methods such as irradiation, to inhibit cell replication, so as to prevent possible tumor formation at the immunization site. Moreover, such methods should not compromise the immunogenicity of the tumor vaccine; rather, they should maintain or when possible enhance its intrinsic immunogenic potential. We recently observed upregulation of the expression of MHC Class I molecules and tumor-associated antigens (TAA) after irradiation of an IL-4 transduced human ovarian carcinoma cell line produced to be used in a phase I immunotherapy trial [12]. This observation has directed our interest in studying the effects of high doses of gamma irradiation on the expression of MHC and other surface antigens in several ovarian carcinoma cell lines.

Our studies show that high doses of gamma irradiation induce a significant and long-lasting upregulation of all surface antigens expressed on the ovarian carcinoma cell lines prior to irradiation. The possible implications of such findings in light of the recent proposed use of genetically modified tumor cells as vaccines for the treatment of women with advanced ovarian cancer will be discussed.

MATERIAL AND METHODS

Tumor cell lines. Four human epithelial ovarian carcinoma cell lines (UCI-101, UCI-107, SKOV-3, and T-222) were used for this study. UCI-101 and UCI-107 have been previously characterized [13, 14] and were kindly provided by Dr. Alberto Manetta, University of California, Irvine. SKOV-3 was purchased from American Type Culture Collection (ATCC), while T-222 was kindly provided by Dr. Benjamin Bonavida, University of California, Los Angeles. All tumor cell lines were maintained at 37°C, 5% CO_2 in complete media (CM) containing RPMI 1640 (Gibco Life Technologies, Grand Island, NY) 10% fetal bovine serum (FBS, Gemini Bioproducts, Calabasas, CA).

High-dose gamma irradiation. Tumor cell lines were irradiated with 5000 and 10,000 cGy in 15-ml conical tubes in CM at room temperature with gamma rays (Cesium 137) at a dose rate of 200 cGy/min. Immediately after irradiation, cells were seeded into T 75 tissue culture flasks (Corning, Corning, NY) in CM and cultured at 37°C in a 5% CO_2 atmosphere. Spent media were changed every other day. Irradiated cells were harvested at Days 2 and 6 for analysis of surface antigen expression by FACS analysis.

Indirect immunofluorescence and flow cytometry. Cells were harvested with 0.25% trypsin in HBSS (Gibco) and washed once in CM. Cell suspensions were counted and distributed into 12 × 75-mm tubes at 5×10^5 cells/tube. Mouse monoclonal antibodies [anti-HLA Class I (mAb W6/32; Accurate Chemical and Scientific Corp., Westbury, NY); anti-HLA Class II (mAb CR3-43; Accurate Chemical and Scientific Corp.); anti-ICAM-1 (mAb LB-2; Becton–Dickinson); anti-CA 125 (mAb OC125; Signet Laboratories, Dedham, MA); anti-HER-2/neu p185 (mAb TA-1; Oncogene Science, Uniondale, NY)] were diluted in cold assay buffer (PBS, pH 7.2, supplemented with 0.1% FBS) and added in a 50-μl volume. A mouse IgG preparation (mAb IgG_2a; Becton–Dickinson) was used as a negative control. Tubes were incubated for 30 min followed by two washes with assay buffer. Phycoerythrin-conjugated goat anti-mouse IgG $F(ab')_2$ (H&L) (Leinco Technologies, St. Louis, MO) diluted 1:100 in assay buffer was added to the cells and the tubes were incubated for 30 min. Following two washings in assay buffer, the final cell pellet was resuspended in 500 μl assay buffer for subsequent analysis. Cells were analyzed with a fluorescence-activated cell sorter (FACS; Becton–Dickinson) [15] with a 15-mW argon laser with an excitation of 488 nm. Fluorescent signals were gated on the basis of for-

ward and right-angle light scattering to eliminate dead cells and aggregates from analysis. Gated signals (10^4) were detected at 585-bp filter and analyzed using Lysis II software.

Statistical analysis. Significance analysis was performed using a paired Student's *t* test.

RESULTS

Expression of surface antigens on ovarian carcinoma tumor cell lines. Flow cytometric analysis of MHC Class I and Class II antigens, ICAM-1, and the TAA CA 125 and Her2-neu antigens was performed on four continuous ovarian cell lines. The results shown in Table 1 indicate that MHC Class I antigens were expressed at variable levels (range of MFI from 41.8 to 834.3) on all four cell lines. Her2-neu antigen was expressed also in all four cell lines (range of MFI from 21.5 to 224.3). In contrast, Class II antigens and CA 125 were not expressed in any cell line. ICAM-1 was expressed in only two cell lines (i.e., UCI-101 and T-222) (range of MFI from 43.8 to 92.2).

Expression of surface antigens after high-dose gamma irradiation. Cell surface antigen expression on the four established cell lines was evaluated 2 days after irradiation and the results were compared to the level of expression on unirradiated control cells. As shown in Figs. 1–3, irradiation with 10,000 cGy caused a significant upregulation of all the surface antigens expressed prior to irradiation. The induction indices for MHC Class I ranged from 1.42 to 2.62, for ICAM-1 from 1.74 to 2.20, and for Her2-neu from 1.22 to 1.71, respectively. It is interesting that irradiation did not induce neoexpression of antigens previously not present on these cells, such as MHC Class II. In one cell line (i.e., T-222), irradiation-induced upregulation was also noted at 5000 cGy and was essentially identical to that found at 10,000 cGy. However, because 5000 cGy was not a sufficient dose to totally block cell replication in some cell lines, we performed the majority of our studies using 10,000 cGy, a dose which consistently blocked cell replication in all cell lines.

Kinetics of expression of surface antigens after high-dose gamma irradiation. Kinetics studies were performed to determine whether the radiation induced of surface antigens was persistent or was a transient response by the tumor cells. Thus, tumor cells from the T-222 cell line were irradiated with 10,000 cGy and analyzed for antigen expression 2 and 6 days after irradiation. As seen in Fig. 4, irradiation of these cells caused increased expression of all the surface antigens at Day 2 as well as Day 6 when compared to the levels expressed by the unirradiated parental cells. Viability studies (trypan blue exclusion) showed that by Day 2 the viability of the irradiated cells was approximately 90% and this dropped to about 30% by Day 6 after 10,000 cGy. Additional studies showed that the enhancement of antigen expression was persistent until all cells died (data not shown).

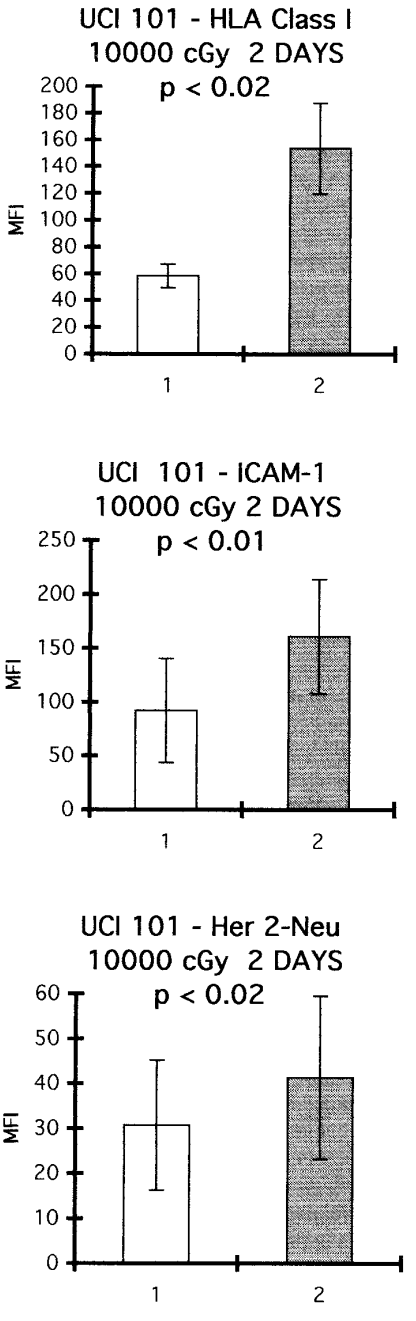

FIG. 1. Expression of surface antigens on UCI-101 ovarian cancer cells prior to and after 10,000 cGy of gamma irradiation. 1, unirradiated cells; 2, irradiated cells.

DISCUSSION

The importance of surface antigen expression and immune recognition has been underscored by studies on human tumor

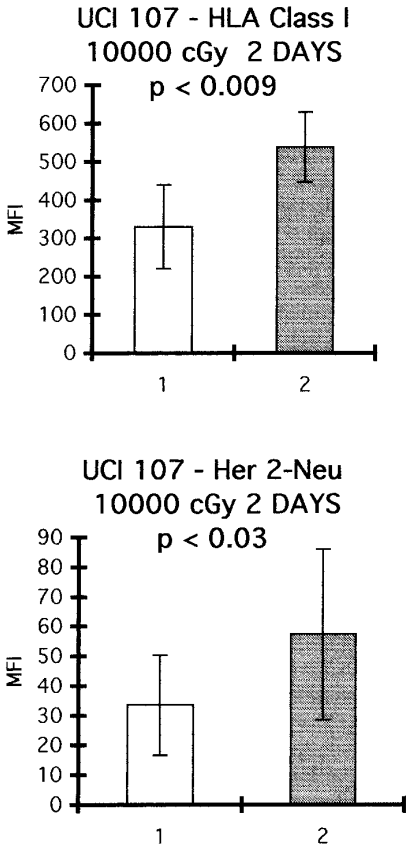

FIG. 2. Expression of surface antigens on UCI-107 ovarian cancer cells prior to and after 10,000 cGy of gamma irradiation. 1, unirradiated cells; 2, irradiated cells. Note that UCI-107 cells do not express MHC Class II, ICAM-1, or Ca 125 surface antigens and these were not induced after irradiation.

cells exposed to TNF-α plus IFN-γ [4, 5, 16]. Such studies have shown that the induction of cytotoxic effector cells requires a higher level of antigen expression than cytotoxic interaction. In fact, tumors not previously considered to be immunogenic but so induced to express higher levels of MHC antigens appear to recruit high-avidity T cells, which can then recognize target cells which express virtually undetectable levels of these surface antigens [4, 5]. Moreover, in studies on melanoma [5, 6] the expression of ICAM-1 antigens in different melanoma clones correlated with the susceptibility of the tumor cells to lysis by specific and nonspecific T-cell clones and, more importantly, upregulation of these antigens, by treatment with interferon-γ in low expressor clones, could boost tumor lysability only by specific, TCR-dependent and HLA-restricted effector cells. In such studies, the relative surface expression of ICAM-1 was a key factor for the outcome of the interaction with T cells and only those clones that expressed these molecules at levels high enough for an efficient interaction with antigen-specific T cells were able to trigger lysis.

Irradiation, together with surgery and chemotherapy, represents one of the most commonly used, standardized, and effective modalities for contemporary cancer therapy. However, in a significant number of cases where whole-body irradiation with fractionated low doses is used (i.e., malignant lymphoma) the efficacy of the therapy cannot be explained merely by the lethal effects of radiation on the tumor cells [17]. There may be additional reasons to explain these findings. Reports dating more than two decades ago note an increased infiltration of lymphocytes surrounding tumor cells following radiation therapy, suggesting that such cells may have become more immunogenic [18, 19]. Most reports evaluating the phenotype of such tumor-infiltrating cells have shown that the majority of these cells are CD 4^+ helper inducer T cells [20]. Cameron *et al.* [21] reported that local tumor irradiation synergizes with tumor-infiltrating lymphocytes (TIL) and IL-2 administration in mediating the regression of established macrometastases. Such results support the possibility that the synergy observed between local irradiation and the systemic administration of IL-2 and TIL cells may be due in part to the upregulated expression of cell surface antigens on the irradiated tumor cells. Indeed, such

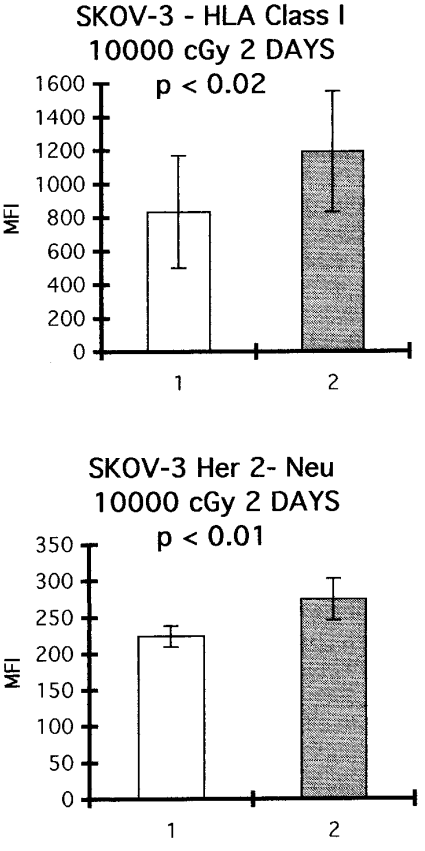

FIG. 3. Expression of surface antigens on SKOV-3 ovarian cancer cells prior to and after 10,000 cGy of gamma irradiation. 1, unirradiated cells; 2, irradiated cells.

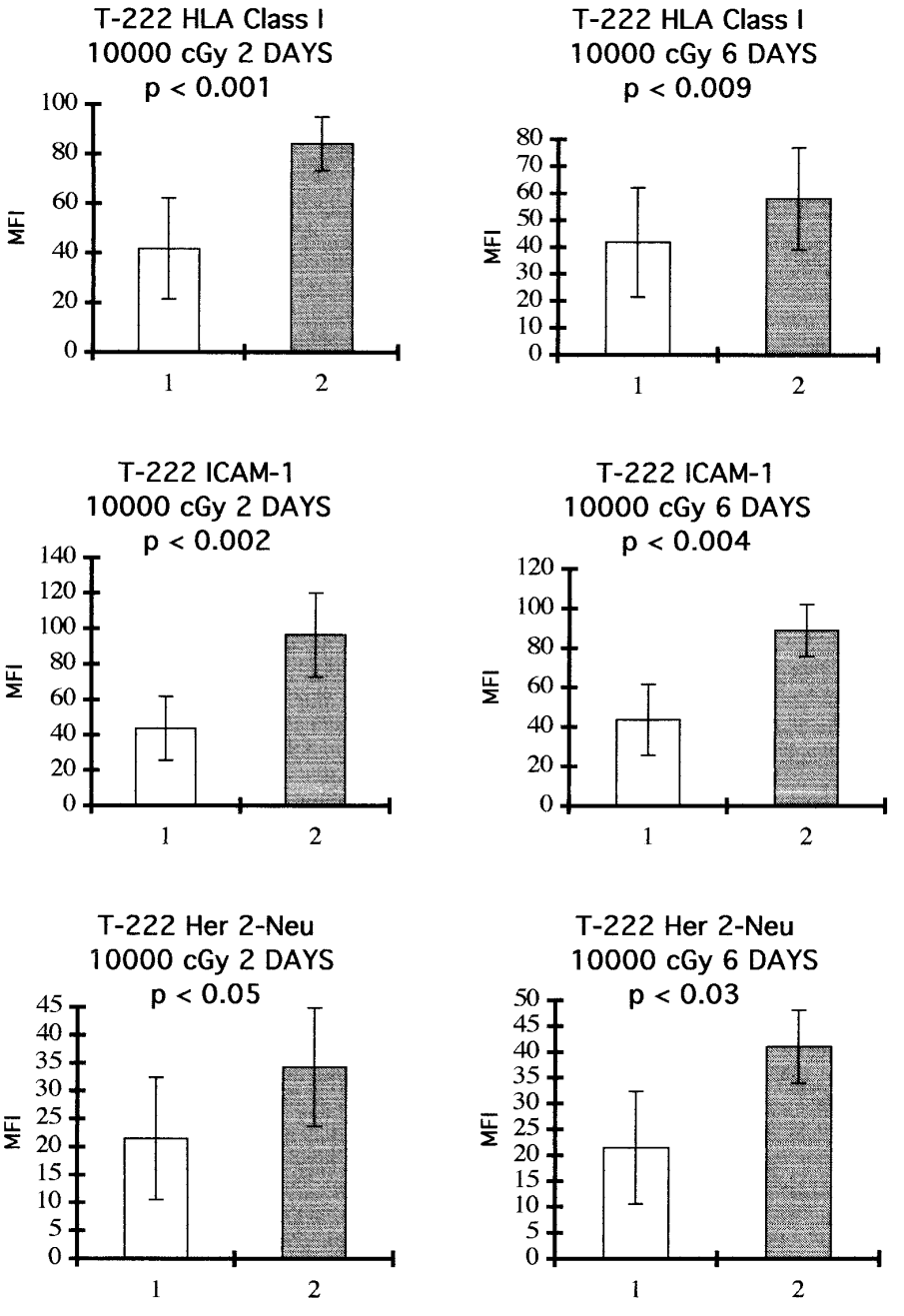

FIG. 4. Kinetics of expression of surface antigens following exposure to high-dose gamma irradiation. T-222 cells were irradiated with 10,000 cGy and analyzed for surface antigen expression 2 and 6 days after treatment. 1, unirradiated cells; 2, irradiated cells.

a phenomenon would render these cells more recognizable by the IL-2-activated, MHC Class I-restricted cytotoxic T lymphocytes. However, adequate documentation of the effects of irradiation on surface antigen expression is lacking.

Further studies documenting the effects of irradiation on tumor immunogenicity were recently reported by Dranoff *et al.* and Huang *et al.* [7, 8] who showed that immunogenicity of irradiated tumor cells was increased and would protect animals against a subsequent challenge of viable parental cells, even though the unirradiated tumor cells were considered to be poorly immunogenic. Such data support the concept that following irradiation important cell surface changes occur which alter the intrinsic immunogenicity of the tumor cells. In addition to these findings, the necessity of blocking cell replication in any human clinical immunotherapy trial using whole-cell tumor vaccines has directed our interest

in studying the phenotypic effect of high doses of gamma irradiation on surface antigen expression on ovarian carcinoma cell lines.

In this regard, few studies have described the phenotypic changes in surface antigen expression following high-dose irradiation [10, 11], and no reports have studied these effects using ovarian cancer cells. Our study investigated the effects of irradiation on the expression of MHC, ICAM-1, and the TAA CA 125 and Her 2-Neu on four established cell lines of human ovarian epithelial tumors. Our results indicate that in all tumors evaluated, a statistically significant increase in the expression of the surface antigens expressed prior to irradiation was consistently noted. This upregulation of surface antigens was noted not only for MHC and ICAM-1 antigens but also for the TAA expressed by the cells before irradiation. When the expression of surface antigens was evaluated at different time points (2 and 6 days after irradiation), this upregulation was found to be persistent. It is interesting that irradiation was unable to induce expression of antigens (such as MHC Class II and ICAM-1) not previously expressed by the unirradiated tumor cells. Taken together, these results indicate that gamma irradiation upregulates the expression of tumor cell surface antigens in a nonspecific manner and that the enhanced expression is persistent until the cells die. Such results are in marked contrast to the differential induction of surface antigens on ovarian carcinoma cell lines by IFN(s) [22] and with the known capacity of these cytokines to induce expression of neoantigens (i.e., MHC II, ICAM-1). These data indicate that distinct pathways of antigen expression are regulated by irradiation or cytokines and that the combination of the two methods could dramatically increase the antigenic expression of such tumor cells and presumably their immunogenicity.

Until common tumor antigens are identified at the genetic level, and the prevalence and biorelevance of these antigens are assessed, whole tumor cells remain the only source of specific tumor antigens for cancer immunotherapy. It is our opinion that irradiation combined with other methods such as exposure to specific cytokines could result in an important increase in the expression of surface antigens in preparation of tumor cells to be used as tumor vaccines. The future design and implementation of clinical trials in this regard will ultimately determine the validity of this approach.

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