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Advances in Brief

Dominant Nature of the Resistance to Fas- and Tumor Necrosis Factor-α-mediated Apoptosis in Human Prostatic Carcinoma Cell Lines[1]

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Abstract

We have recently found (O. W. Rokhlin *et al.*, Cancer Res., *57:* 1758–1768, 1997) that, although Fas ligation induced apoptosis in two of six human prostatic carcinoma cell lines investigated, the apoptotic machinery involved in Fas-mediated killing is already in place in Fas-resistant cell lines. Here, we investigated Fas- and tumor necrosis factor-α (TNF-α)-mediated apoptosis in cell hybrids between resistant (DU145 and JCA1) and sensitive (ALVA31 and PC3) cell lines. All three types of hybrid cells investigated, F1(DU145 × PC3), F1(JCA1 × PC3), and F1(JCA1 × ALVA31), were found to be resistant to Fas- and TNF-α-mediated apoptosis at the same level as the corresponding parental resistant cell lines. These results indicate that resistance to Fas- and TNF-α-mediated apoptosis dominates over sensitivity in cell hybrids and suggest that resistance may be regulated by an apoptosis suppressor factor or factors acting in resistant but not in sensitive cells.

Introduction

We have recently reported that all six human prostatic carcinoma cell lines examined (ALVA31, DU145, JCA1, LNCaP, ND1, and PC3) expressed Fas antigen (1). However, agonistic anti-Fas monoclonal antibody induced apoptosis in only two of six cell lines. In addition, treatment with CHX[3] converted the phenotype of resistant cell lines from Fas resistant to Fas sensitive. Further, anti-Fas treatment of both resistant and sensitive cell lines induced rapid tyrosine phosphorylation or dephosphorylation of multiple proteins. These results indicate that the apoptotic machinery involved in Fas-mediated apoptosis is already in place in both resistant and sensitive cell lines.

Our working hypothesis is that resistance to Fas-mediated apoptosis is dependent on the presence of a labile regulatory protein or proteins. Identification of this factor or factors is critical both for understanding the mechanism(s) of Fas-mediated apoptosis and for the development of specific therapeutic approaches to sensitize prostatic carcinomas to anticancer therapy. It should be possible to apply expression selection of cDNA from expression libraries to identify the gene(s) that determine the difference in sensitivity of prostatic carcinoma cell lines to Fas-mediated apoptosis. However, the application of such an approach requires the determination of a dominant or recessive nature of the studied phenotype. Although preliminary experiments with CHX suggest the existence of a dominant suppressor or suppressors of apoptosis, this hypothesis has to be tested directly. To this end, we generated somatic hybrids between Fas-resistant (DU145 and JCA1) and Fas-sensitive (ALVA31 and PC3) cell lines. We show here that hybrid cells (F1) are resistant to Fas-mediated apoptosis, similar to the Fas-resistant parental cell lines. Thus, resistance to Fas-mediated apoptosis dominates over sensitivity in cell hybrids. Therefore, resistance is likely to be regulated by an apoptosis suppressor factor or factors acting in resistant but not in sensitive cells. We also investigate the sensitivity of parental and hybrid cells to TNF-α-mediated apoptosis and show that hybrid cells express the same phenotype as parental resistant cell lines.

Materials and Methods

The human prostatic cancer cell lines, cell culture, flow cytometric analysis, proliferation assay, quantitative DNA fragmentation assay, CHX treatment of cells, and generation of G418-resistant variants of ALVA31 and PC3 cell lines have been described previously (1).

Cell Fusion. In preliminary experiments, we determined the concentrations of hygromycin that can kill the cell lines DU145 and JCA1 and concentrations of G418 that were toxic for ALVA31 and PC3. DU145 died after 10 days of culture in the presence of 300 μg/ml hygromycin; JCA1 died after 10 days in the presence of 200 μg/ml hygromycin; 800 μg/ml G418 was found to be toxic for ALVA31; and 200 μg/ml G418 was toxic for PC3 after 2 weeks of culture. Transfection of cell lines ALVA31 and PC3 with pRSV.5(neo) vector has been described previously (1). Fas-resistant cell lines DU145 and JCA1 were transfected with vector pRSV.5(hygro), conferring resistance to hygromycin (2). After transfection, cell populations were selected in the presence of hygromycin to obtain stable derivatives that were resistant to the drug. G418-resistant ALVA31 and PC3 were used for fusion with DU145 and JCA1, which were resistant to hygromycin. To prepare the hybrid cells, parental cell lines were harvested by trypsin-EDTA as described previously (1), washed twice with serum-free RPMI 1640, mixed in 1:1 ratio, fused using 50% polyethylene glycol solution, pH 7.2–7.4, and cultured in the RPMI 1640 containing 10% FCS and both selecting agents, hygromycin and G418. Double-resistant hybrid cells were isolated as mass cultures and used in further experiments. We have produced three different types of hybrid cells: F1(JCA1 × PC3), F1(JCA1 × ALVA31), and F1(DU145 × PC3).

Results and Discussion

Hybrid Cells Express Markers of Both Parental Cell Lines. We used flow cytometric analysis to determine whether hybrid cells expressed markers which were characteristic of parental cell lines. As shown in Fig. 1*A*, both CD33 expression, which is characteristic of ALVA31, and CD40 expression, which is characteristic for JCA1, were seen in hybrid cells F1(JCA1 × ALVA31). The levels of CD33 and CD40 cell surface expression in hybrid cells were approximately the same as those in the parental cell lines. Similarly, hybrid cells F1(JCA1 × PC3) expressed both parental cell line markers, CD13 and CD40 (Fig. 1*B*). In addition, we have recently obtained a monoclonal antibody, 5D4, which was found to be specific for prostate tumor cell lines as well as for some other epithelial tumors.[4] As shown in Fig.

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[3] The abbreviations used are: CHX, cycloheximide; TNF-α, tumor necrosis factor-α; mAb, monoclonal antibody; Ab, antibody.

[4] O. W. Rokhlin and M. B. Cohen, unpublished results.

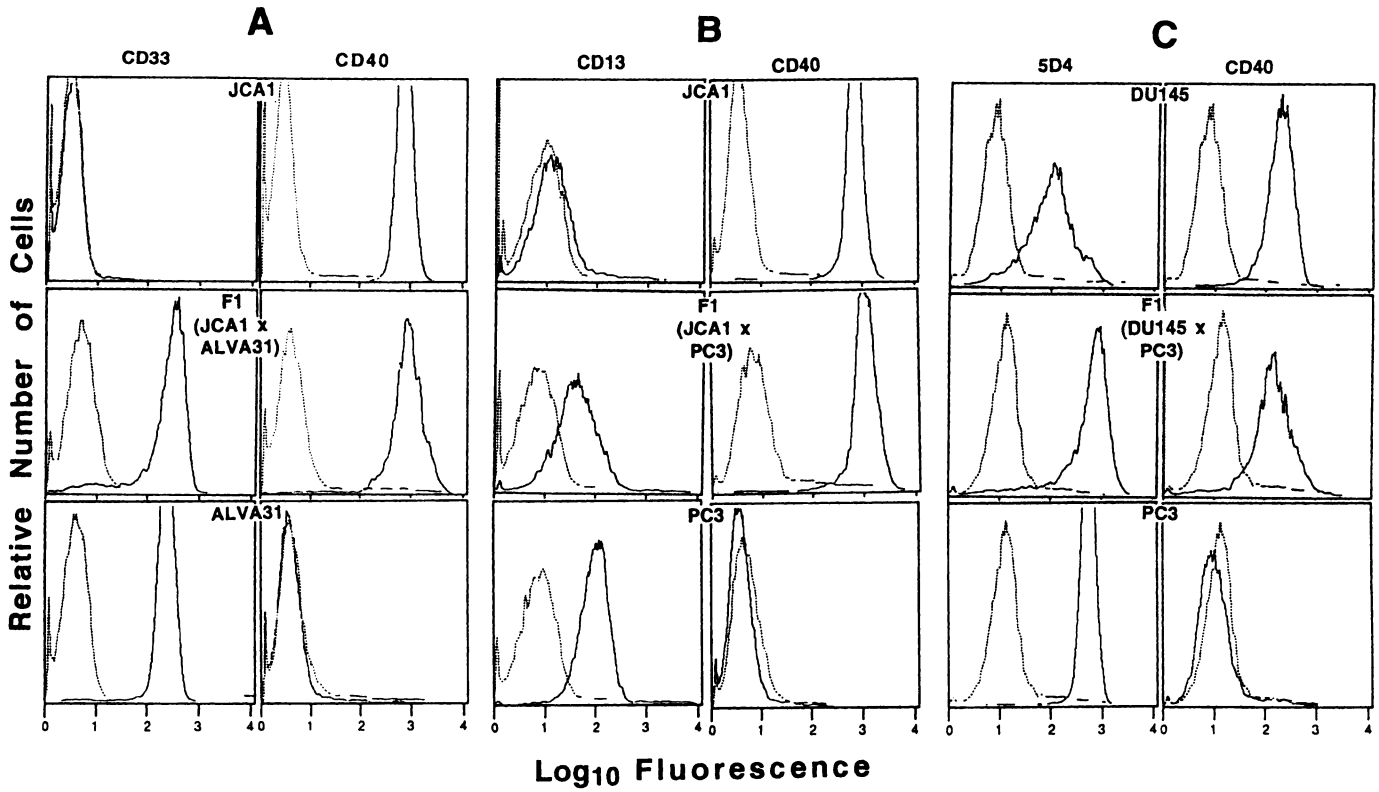

Fig. 1. Reactivity of CD33, CD13, CD40, and 5D4 mAbs with human prostatic carcinoma cell lines was analyzed by flow cytometry. Bound mAbs were detected with phycoerythrin-labeled goat Abs to mouse immunoglobulins. ...., control with irrelevant IgG; —, expression of specific antigens. *A*, analysis of F1(JCA × ALVA31) hybrid cells and parental cell lines; *B*, analysis of F1(JCA1 × PC3) hybrid cells and parental cell lines; and *C*, analysis of F1(DU145 × PC3) hybrid cells and parental cell lines.

1*C*, this antibody reacted much more strongly with PC3 cells than it did with DU145, and hybrid cells F1(DU145 × PC3) expressed 5D4 antigen at the same level as did parental PC3 cells. At the same time, this F1 expressed CD40 marker, which is characteristic for DU145 parental cells. Thus, all three hybrid cell lines were found to express a combination of cell surface markers from both parental cell lines. These results suggest that cell fusion itself does not affect the expression of parental cell lines genes and confirm the hybrid nature of analyzed cells.

Dominant Nature of the Resistance to Fas- and TNF-α-mediated Apoptosis in Hybrid Cells. Previously, we suggested that resistance is dependent on the presence of a specific protein or proteins that determine resistance to Fas-mediated apoptosis. We therefore anticipated that hybrid cell lines should be resistant to Fas-mediated apoptosis. Indeed, as shown in Fig. 2*A*, all three hybrid cell lines were found to be resistant to Fas-mediated growth inhibition and apoptosis at levels similar to those of parental resistant cell lines. We have previously shown that Fas-sensitive cell lines ALVA31 and PC3 were also sensitive to TNF-α-mediated apoptosis and growth inhibition (1). We have now determined that Fas-resistant cell lines DU145 and JCA1 were also resistant to TNF-α treatment (Fig. 2*B*) in both proliferation and apoptosis assays and, more importantly, that all three hybrid cell lines were found resistant to TNF-α treatment.

Because the phenotype of resistant cell lines could be converted from Fas resistant to Fas sensitive under CHX treatment, it was of interest to determine whether the phenotype of hybrid cell lines could be converted in the same way. The data summarized in Table 1 shows that the hybrid cell line F1(DU145 × PC3) is sensitive to Fas-mediated apoptosis under CHX treatment at a level similar to that of the parental (Fas-resistant) cell line DU145. The two hybrid cell lines resulting from fusion between the Fas-resistant cell line JCA1 and the Fas-sensitive cell lines ALVA31 and PC3 were found to be more resistant to Fas-mediated apoptosis under CHX treatment than was the parental cell line (Table 1). It is also worth noting that in the presence of CHX, JCA1 was found to be more

Table 1 *Effects of anti-Fas mAb, TNF-α, and CHX on human prostatic carcinoma cell lines as estimated by quantitative DNA fragmentation assay*[a]

Cell line	CHX (25 μg/ml)	A-Fas[b]	CHX (μg/ml) + A-Fas[b]			TNF-α	CHX (μg/ml) + TNF-α		
			0.25	2.5	25		0.25	2.5	25
DU145	4 ± 3	5 ± 4	12 ± 5	31 ± 7	46 ± 9	7 ± 3	9 ± 5	26 ± 7	45 ± 8
JCA1	5 ± 3	7 ± 3	6 ± 3	39 ± 8	43 ± 8	6 ± 4	1 ± 1	10 ± 4	29 ± 6
F1(JCA1 × ALVA31)	9 ± 4	4 ± 4	6 ± 3	21 ± 5	32 ± 6	4 ± 3	7 ± 4	17 ± 3	28 ± 5
F1(JCA1 × PC3)	12 ± 3	3 ± 3	6 ± 4	20 ± 4	30 + 5	6 ± 5	8 ± 3	15 ± 4	25 ± 6
F1(DU145 × PC3)	12 ± 4	7 ± 3	12 ± 5	22 ± 7	33 ± 5	9 ± 2	16 ± 3	21 ± 3	37 ± 2
ALVA31	16 ± 6	31 ± 6	42 ± 5	58 ± 7	61 ± 6	45 ± 9	39 ± 7	62 ± 9	68 ± 11
PC3	17 ± 7	47 ± 8	61 ± 6	66 ± 7	69 ± 4	35 ± 8	45 ± 9	63 ± 7	63 ± 9

[a] Data are expressed as an arithmetic mean of percentage killing of three different experiments, ±SD. Cells were prelabeled with [^{3}H]thymidine and plated at a density of 3000–6000 cells/well. Anti-Fas Abs, TNF-α, and CHX were added at the time of plating, and cells were harvested 48 h later.

[b] A-Fas, anti-Fas.

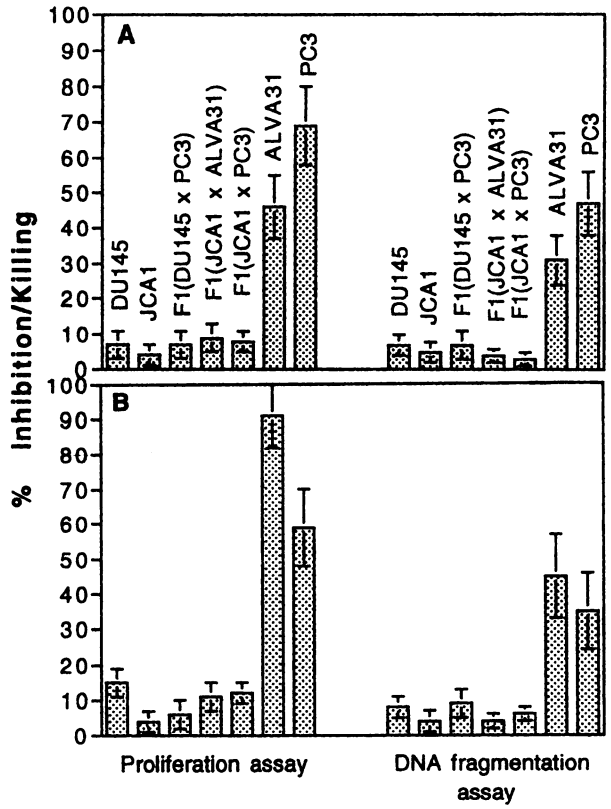

Fig. 2. Assessment of response of hybrid cells and parental cell lines to treatment with anti-Fas mAb (*A*) and TNF-α (*B*) in both proliferation and quantitative DNA fragmentation assays. For proliferation assay, cells were plated at a density of 2000 cells/well in a 96-well flat-bottomed plate; anti-Fas mAb (1 μg/ml) and TNF-α (20 ng/ml) were added at the time of plating. After 32 h of culture, 1 μCi/well [^{3}H]thymidine was added, and cells were harvested 16 h later. For DNA fragmentation assay, cells were prelabeled with [^{3}H]thymidine and plated at a density of 3000–6000 cells/well. Abs and TNF-α were added at the time of plating, and cells were harvested 48 h later. *Columns*, means of five separate experiments; *bars*, SD.

sensitive to Fas-mediated apoptosis than it was to TNF-α-mediated apoptosis. Even the highest concentration of CHX (25 μg/ml) resulted in approximately 25% apoptosis under TNF-α treatment, whereas 2.5 μg/ml CHX induced apoptosis in almost 40% of JCA1 cells under anti-Fas treatment. At the same time, DU145 expressed the same level of sensitivity to both Fas- and TNFα-mediated apoptosis under CHX treatment. There is evidence that apoptosis that is induced by TNF receptor 1 and Fas involves common as well as distinct mechanisms (3). Our results suggest that the resistance to Fas- and TNF-α-mediated apoptosis might be determined by a factors that are common for both pathways in the DU145 cell line, whereas in the case of JCA1, these factors may be different.

In conclusion, we have determined that resistance to Fas- and TNF-α-mediated apoptosis and growth inhibition is dominant in cell hybrids. This result may be explained by the presence of an apoptosis suppressor factor or factors that are active in resistant but not in sensitive cells. Identification of this factor or factors is critical both for understanding the mechanisms of Fas- and TNF-α-mediated apoptosis and for the development of specific therapeutic approaches to sensitize prostatic carcinomas to anticancer therapy. On a broader level, it is worth noting that, to the best of our knowledge, the approach used in this study has not been previously applied. The establishment of cell hybrids, as shown here, may have other applications in understanding apoptosis.

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