S-Nitrosylation of the Death Receptor Fas Promotes Fas Ligand–Mediated Apoptosis in Cancer Cells

LISSBETH LEON-BOLLOTTE,[*,‡,§] SELVAKUMAR SUBRAMANIAM,[*,‡,§] OLIVIER CAUVARD,[*,‡,§] STÉPHANIE PLENCHETTE–COLAS,[*,‡,§] CATHERINE PAUL,[*,‡,§] CINDY GODARD,[*,‡,§] ANTONIO MARTINEZ–RUIZ,[‖] PATRICK LEGEMBRE,[¶] JEAN–FRANÇOIS JEANNIN,[*,‡,§] and ALI BETTAIEB[*,‡,§]

[*]Ecole Pratique des Hautes Etudes, Tumor Immunology and Immunotherapy Laboratory, Dijon, France; [‡]Inserm U866, Dijon, France; [§]University of Burgundy, Dijon, France; [‖]Servicio de Immunologia, Hospital Universitario de La Princesa, Instituto de Investigacion Sanitaria Princesa (IP), c/Diego de Leon 62, E-28006, Madrid, Spain; [¶]Université de Rennes-1, IRSET/EA SERAIC, 2 avenue du Prof Léon Bernard, 35043 Rennes, France

BACKGROUND & AIMS: Fas belongs to the family of tumor necrosis factor receptors which induce apoptosis. Many cancer cells express Fas but do not undergo Fas-mediated apoptosis. Nitric oxide reverses this resistance by increasing levels of Fas at the plasma membrane. We studied the mechanisms by which NO affects Fas function. **METHODS:** Colon and mammary cancer cell lines were incubated with the NO donor glyceryl trinitrate or lipid A; S-nitrosylation of Fas was monitored using the biotin switch assay. Fas constructs that contained mutations at cysteine residues that prevent S-nitrosylation were used to investigate the involvement of S-nitrosylation in Fas-mediated cell death. Apoptosis was monitored according to morphologic criteria. **RESULTS:** NO induced S-nitrosylation of cysteine residues 199 and 304 in the cytoplasmic part of Fas. In cancer cells that overexpressed wild-type Fas, S-nitrosylation induced Fas recruitment to lipid rafts and sensitized the cells to Fas ligand. In cells that expressed a mutant form of Fas in which cysteine 304 was replaced by valine residue, NO-mediated translocation of Fas to lipid rafts was affected and the death-inducing signal complex and synergistic effect of glyceryl trinitrate–Fas ligand were inhibited significantly. These effects were not observed in cells that expressed Fas with a mutation at cysteine 199. **CONCLUSIONS: We identified post-translational modifications (S-nitrosylation of cysteine residues 199 and 304) in the cytoplasmic domain of Fas. S-nitrosylation at cysteine 304 promotes redistribution of Fas to lipid rafts, formation of the death-inducing signal complex, and induction of cell death.**

Keywords: Colon Cancer; Tumor; Signaling; Localization.

Fas (APO-1/CD95) is a transmembrane receptor that belongs to the tumor necrosis factor–receptor superfamily. Fas plays a physiological role in immune response control (eg, through mediating activation-induced T-cell death).[1,2] Interaction of the Fas receptor with its ligand (FasL) initiates assembly of an intracellular death-inducing signaling complex (DISC)[3] that contains the Fas-associated death domain (FADD) adaptor protein and either procaspase-8 or procaspase-10.[4,5] Autoprocessing of these proenzymes within the DISC generates proteolytically active enzymes.[6] In type I cells, in which Fas is localized mainly in plasma membrane lipid rafts,[7–10] active caspase-8 directly activates a cascade of effector caspases in a mitochondria-independent manner. In type II cells, whose rafts do not contain Fas, limited amounts of caspase-8 are activated at the DISC and cleave the proapoptotic Bcl-2 family member Bid. In turn, truncated Bid moves to the mitochondria to stimulate the release of soluble factors that activate the caspase cascade in the cytosol.[11,12]

Engagement of Fas can trigger apoptosis of some cancer cells[13,14] but not all cancer cells respond to FasL. Changes of Fas expression frequently are found in cancer mutations in the Fas gene,[15,16] down-regulation of Fas expression at the cell surface,[17] and high levels of anti-apoptotic proteins such as FADD-like interleukin-1-beta-converting enzyme (FLICE)-like inhibitory protein; Bcl-2 and inhibitors of apoptosis[18–21] can decrease the potency of the Fas-mediated apoptotic signal. A series of post-translational modifications also modulate Fas-dependent cell death. The membrane-proximal region of the cytoplasmic domain of Fas can be phosphorylated by an unidentified kinase.[22,23] The receptor also may be palmitoylated at cysteine 199 (Cys199) in human beings and Cys194 in mice, and this facilitates Fas redistribution into lipid rafts and its association with ezrin and the actin cytoskeleton.[24,25] Cys294 of murine Fas can be S-glutathionylated after FasL engagement, which involves caspase-dependent degradation of glutaredoxin 1, and this promotes aggregation of the receptor, its recruitment into lipid rafts, and propagation of Fas-dependent

Abbreviations used in this paper: ALPS, autoimmune lymphoproliferative syndrome; Cys, cysteine; DISC, death-inducing signaling complex; FADD, Fas-associated death domain; FasL, Fas-ligand; FLICE, FADD-like interleukin-1-beta-converting enzyme; GFP, green fluorescent protein; GTN, glyceryl trinitrate; NO, nitric oxide; NOS, nitric oxide synthase; SNO, nitrosothiol; SNO-proteins, S-nitrosylated proteins; 3D, 3-dimensional; WT, wild-type.

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apoptosis.[26,27] Less Fas accumulates at the surface of cancer cells, which have a reduced apoptotic response to FasL.[28–30] Fas expression is positively regulated by the transcription factor nuclear factor-κB,[31] whereas other transcription factors such as activator protein-1, signal transducer and activator of transcription 3,[32] Yin Yang 1,[33] and the Fas-associated phosphatase-1[34,35] have the opposite effect.

The down-regulation or mutation of Fas has been proposed as a mechanism by which cancer cells avoid destruction by the immune system through reduced apoptosis sensitivity.[36] Breaking such resistance was rendered possible with some anticancer drugs that enhance Fas-receptor expression and aggregation at the surface of tumor cells, thereby increasing the apoptotic response to FasL.[36–38] We have shown that nitric oxide also has a sensitizing effect by increasing Fas expression on cancer cells,[39] which raises the possibility that Fas-receptor expression or function could be regulated by S-nitrosylation, especially because Fas harbors a 3-dimensional (3D)

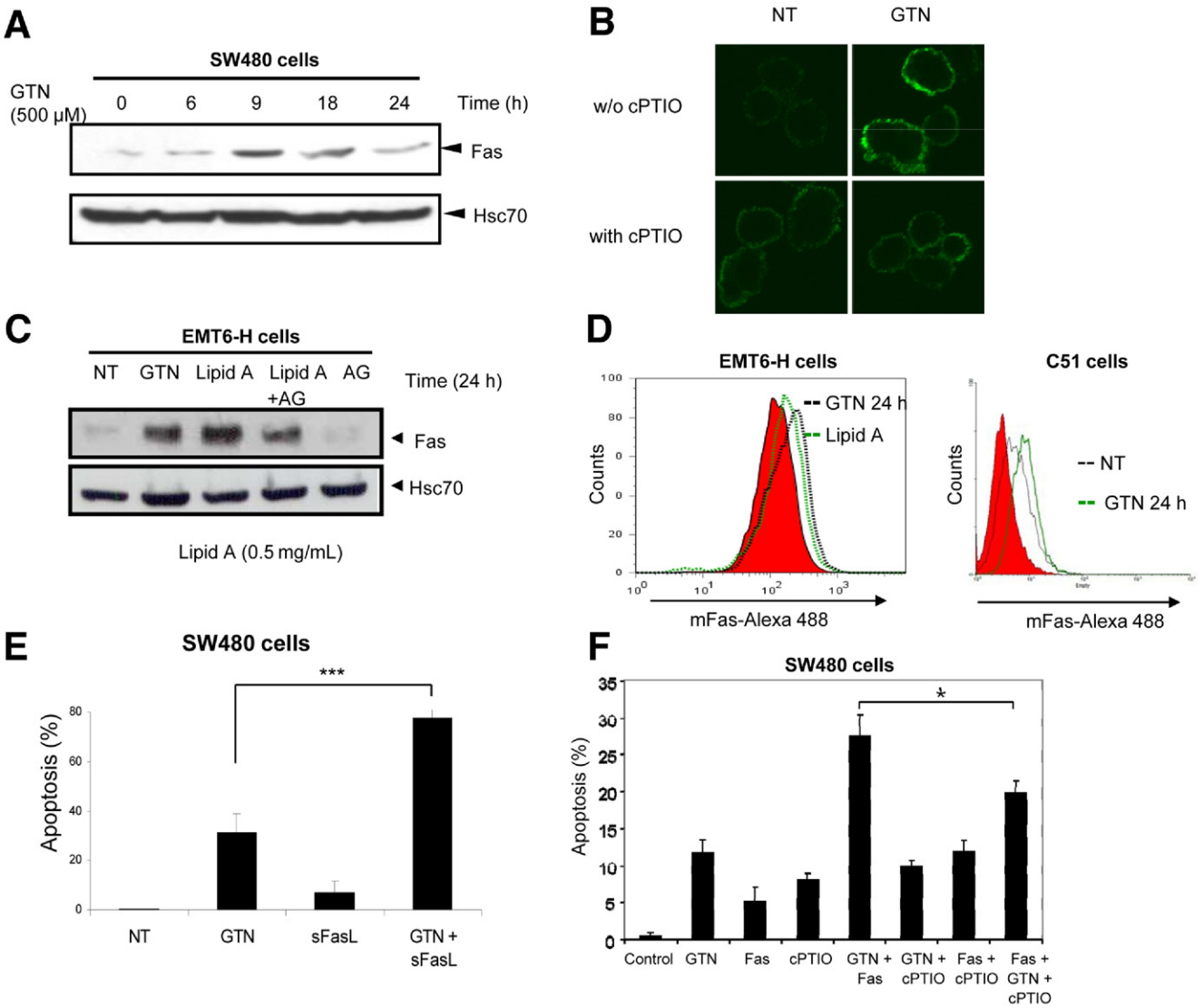

Figure 1. NO increases Fas expression and sensitizes cells to FasL-mediated apoptosis. Colon cancer cells SW480 and C51 and mammary cancer cells EMT6-H were left nontreated (NT) or treated with 500 mmol/L GTN or with 0.5 mg/mL lipid A for the indicated times. (*A* and *C*) For analysis of whole-cell Fas expression, cells were lysed and 50 μg of each lysate was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblot using the anti-Fas antibody. Constitutive heat shock protein 70 (Hsc70) was used as loading control. (*C*) To verify that any increase in Fas expression in lipid A–treated EMT6-H cells involved inducible NOS activity and hence NO, cells were treated with the NOS inhibitor aminoguanidine (AG). Cell surface expression of Fas was evaluated using a mouse anti-Fas antibody by (*B*) confocal microscopy in SW480 cells or by (*D*) flow cytometry in EMT6-H and C51 cells. Data are representative of at least 3 independent experiments. (*E*) Apoptosis was induced in SW480 by treatment with 500 μmol/L GTN for 24 hours then with or without soluble FasL (sFasL) for 24 hours. (*F*) To verify that apoptosis induced by GTN and FasL involved NO, SW480 cells were treated with the NO scavenger 2-(4-Carboxyphenyl)-dihydro-tetramethyl-imidazol-oxide (cPTIO) and GTN for 24 hours then with or without sFasL for 6 hours. This short treatment with sFasL (6 vs 24 h) is owing to the high toxicity of cPTIO. Apoptotic cells were identified by fluorescence microscopy after staining nuclei with Hoechst 33342 and then counted. Data are the ± standard error of the mean of at least 3 independent experiments. *$P < .05$ and ***$P < .001$.