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Targeting a Novel Onco-glycoprotein Antigen at Tumoral Pancreatic Cell Surface by mAb16D10 Induces Cell Death

Lydie Crescence,*† Evelyne Beraud,*† Veronique Sbarra,*† Jean-Paul Bernard,*†‡ Dominique Lombardo,*†‡ and Eric Mas*†‡

The mAb16D10 was raised against a pathological onco-glycoform of bile salt-dependent lipase isolated from the pancreatic juice of a patient suffering from a pancreatic adenocarcinoma. We previously showed that mAb16D10 specifically discriminates human pancreatic tumor tissues from other cancer and nontumor tissues. In this study, we report that mAb16D10 inhibited the proliferation of only human pancreatic tumor cells expressing 16D10 plasma membrane Ag. Interaction of mAb16D10 with its cognate surface Ag on pancreatic cells promoted cell death by activation of the p53- and caspase-dependent apoptotic pathway, and silencing of p53 decreased cell death. The decreased proliferation was also partly due to cell cycle arrest in G1/S phase, mAb16D10 triggering of glycogen synthase kinase-3β (GSK-3β) activation, degradation of β-catenin, and decreased expression of cyclin D1. GSK-3β positively affected p53 expression in pancreatic tumor cells after mAb16D10 binding. Inhibition of GSK-3β activity reversed the effects induced by mAb16D10 in SOJ-6 cells, supporting the pivotal role of GSK-3β signaling in the mechanisms of action induced by mAb16D10. Also, mAb16D10 cell treatment led to membrane overexpression of E-cadherin. Both E-cadherin and tumor Ag were localized in membrane lipid cholesterol-rich microdomains and are thought to belong to signaling platforms involved in the induction of cell cycle arrest and cell death. Overall, this study reveals that mAb16D10 holds great potential to prevent pancreatic tumor proliferation by apoptotic cell death, thus promising therapeutic prospects for treatment of pancreatic adenocarcinoma, a highly lethal disease. The Journal of Immunology, 2012, 189: 3386–3396.
cells in vitro and next to elucidate the intracellular pathways involved in this process. We find that this novel Ab, which targets a pancreatic glycoprotein, directly induces tumor cell apoptosis with a central involvement of GSK-3β signaling.

Materials and Methods
Reagents and Abs
Patented mAb16D10 (IgM), which recognizes the O-glycosylated C-terminal domain of tumor BSDL, was homemade (17). Reagents were from these sources: Abs to Bax (sc-6236) and E-cadherin (I80) (Santa Cruz Biotechnology, Santa Cruz, CA); Abs to cleaved caspase-3 (Asp175), caspase-3, cleaved caspase-7 (Asp198), caspase-7, cleaved caspase-9 (Asp330), caspase-9, caspase-8, cleaved poly ADP-ribose polymerase 1 (PARP1) (Asp214), PARP1, Bcl-2, phospho-β-catenin (Ser33/37, Thr41) (Cell Signaling Technology, Beverly, MA); Abs to caspase-8 (Abcam, Cambridge, U.K., and Cell Signaling Technology); polyclonal Abs to β-catenin (Abcam); peroxidase-conjugated goat anti-rabbit IgG and anti-mouse IgG (respectively Cell Signaling Technology and Calbiochem, San Diego, CA); Alexa-conjugated goat anti-rabbit IgG, anti-mouse IgG and IgM (Invitrogen, Cergy-Pontoise, France); Abs to β-actin, irrelevant mouse IgM, FITC-conjugated goat anti-mouse IgM, fumonisin B1 and B2, L-cycloserine, conjugated goat anti-rabbit IgG, anti-mouse IgG and IgM (Invitrogen, 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), methyl-

Expression of p53 mRNA induced by mAb16D10
Total RNA was isolated using TRIzol reagent (Invitrogen), and RT-PCR was performed as described (18). p53 amplification was performed using a pair of primers: forward, 5′-ATGGAGGAGCCGCAGTCAATGCACCTGAC-3′; reverse, 5′-TCAGCTGAGTCGGCCTTCTGTTCTGA-3′. Some specific PCR products were purified from agarose gel using the PCR Clean-up Gel Extraction kit (Macherey-Nagel) and sequenced by Genome Express (Meylan, France).

p53-targeted small interfering RNA treatment
SOJ-6 cells were transfected with p53-targeted small interfering RNA (siRNA) 1 and 2 (SignalSilence p53 siRNA Kit; Cell Signaling Technology) using Lipofectamine 2000 (Invitrogen). Transfection medium was replaced after 6 h with fresh 10% FCS medium before addition of mAb16D10 for 24 h.

Cell cycle analysis
DNA contents were analyzed using PI as described (20). Cell cycle phase distribution was detected by flow cytometry and analyzed by ModFit Lite software (Verity Software House, Topsham, ME). The red fluorescence of single event was recorded to measure the DNA index.

SDS-PAGE and Western blotting
PAGE and Western blot analysis were performed as described (17). Membranes were developed with a chemiluminescent substrate (Roche Diagnostics).

Statistical analysis
All data are mean ± SD. Significant differences among the groups were determined by unpaired Student t test. p values < 0.05 were considered significant.

Results
mAb16D10 decreases the number of living cells of only human pancreatic tumor line expressing surface 16D10 Ag
SOJ-6 and BxPC-3 line cells expressed 16D10 Ag at their surface, as assessed by flow cytometry analyses, whereas PANC-1 cells did not (Fig. 1A). mAb16D10 reactivity decorated the plasma membranes of the former cells, shown by fluorescence microscopy (Fig. 1B). Binding of mAb16D10 to its receptor led to internalization (data not shown). We next evaluated its ability to affect the viability of tumor cells in vitro. mAb16D10 significantly induced a decrease of mitochondrial dehydrogenase activity—an indicator of the number of living cells—of SOJ-6 and BxPC-3 cells in a dose-dependent manner (Fig. 2A). This decrease clearly became more important at 48 h (data not shown). In parallel, relative PI-positive SOJ-6 and BxPC-3 cells increased in a dose- and time-dependent manner (data not shown). In further experiments, mAb16D10 was used at 25 μg/ml, which is close to the IC50. Moreover, we observed SOJ-6– and BxPC-3–decreased cell viability determined by blue trypan exclusion after treatment with mAb16D10 (Fig. 2C, 2D). Notably, the tumor cells, which have escaped death in the early phase, finally died by 72 h. In contrast, mAb16D10 had no effects on the viability of PANC-1 cells, whose plasma membranes were not reactive to this Ab (Fig. 2A, 2E). The treatment of pancreatic tumor cells with irrelevant Abs did not significantly affect their growth (Fig. 2B). Altogether, these results indicate that the decrease of pancreatic tumor cell viability was most likely mediated by the interaction between mAb16D10 and its cognate surface Ag.

mAb16D10 evokes cell death and induces caspase activation
We evaluated whether the decrease in cell viability and proliferation upon cell treatment with mAb16D10 was due to cell death. We therefore studied morphological changes of the nucleus. Chromatin condensation was observed upon treatment of SOJ-6 and BxPC-3 cells with mAb16D10 (Fig. 3A, arrows), contrary to mAb16D10-treated PANC-1 cells. The nucleus showed no morphological changes upon

Cell viability assay
Mitochondrial succinate dehydrogenase activity, an indicator of the number of viable cells, was determined by MTT assay (19). Cells were cultured in 96-well culture plates for 24 h with 10% FCS and increasing concentrations of mAb16D10. Colorimetric signal was measured at 570 nm. Cell viability was also determined by trypan blue exclusion after treatment with mAb16D10 at 0, 24, and 48 h. Independent experiments were performed at least three times; each point was replicated eight times.

Caspase activities
Cells were treated with Abs in their respective medium with 10% FCS for 24 h before the addition of CaspACE FITC-VAD-FMK In Situ Marker according to the manufacturer’s instructions (Promega). Upon caspase action on FITC-VAD-fmk, cells become fluorescent; these cells were counted on 10 fields randomly examined under fluorescence microscopy in triplicate.
SOJ-6 and BxPC-3 cell treatment with irrelevant Abs (Fig. 3A). We investigated whether the cell death promoted by mAb16D10 involved activation of cell death effector caspases. Caspase activation, monitored by cell fluorescence, was significantly increased in SOJ-6 and BxPC-3 cells after treatment with mAb16D10 compared with that in untreated cells and irrelevant Abs-treated cells (Fig. 3B). No increase in fluorescent cells was detected in mAb16D10-treated PANC-1 cells compared with controls. Furthermore, mAb16D10 induced a time-dependent increase in caspase enzymatic activities in SOJ-6 and BxPC-3 cells, with maximal numbers of fluorescent cells obtained at 24 h (Fig. 3C). To identify the caspases involved in this process, their cleavage was investigated upon treatment of SOJ-6 cells with mAb16D10. Caspase-9, caspase-3, and caspase-7 were cleaved consecutively to mAb16D10 cell treatment, as assessed by immunoblotting (Fig. 3D). In contrast, no cleavage of caspase-8 was observed (Fig. 3E) using two different specific Abs. An ultimate effector that is caspase-3– and caspase-7–dependent is PARP1. Inactivation of PARP1 resulted from its cleavage into fragments of 85 and 24 kDa. These fragments were detected in lysates of SOJ-6 cells after treatment with mAb16D10 (Fig. 3D). These biochemical changes associated with cell death support the notion that cell treatment with mAb16D10 evoked cell death via the caspase-3–, caspase-7–dependent pathway.

**mAb16D10 induces p53 expression and affects Bax and Bcl-2 expression**

Because p53 tumor suppressor acts in proliferation, apoptosis, cellular senescence, activation of DNA repair, and cell cycle arrest (21–24), we investigated its expression in SOJ-6 cells with and without Ab treatment. p53 was detected in SOJ-6 cells treated with mAb16D10.
but not in control SOJ-6 cells, untreated or treated with irrelevant Abs (Fig. 4A). This result was confirmed at the transcriptional level by RT-PCR using specific probes for p53 (Fig. 4B). Because the p53 gene is mutated in up to 50% of pancreatic adenocarcinoma, RT-PCR p53 transcripts of SOJ-6 cells were sequenced. We found a sequence coding for Arg72, which agrees with a report on the existence of two p53 isoforms with Arg or Pro at position 72 (25, 26) and one mutation of Tyr236 to Asp236. To determine whether mutated p53 yielded functional proteins in relation to the caspase-dependent death pathway, we silenced its expression in SOJ-6 cells with two p53-targeted siRNAs. siRNA 2 was more efficient than siRNA 1 in inhibiting p53 expression (Fig. 4B). Next, to evaluate mutated p53 functionality, we assessed caspase activity in SOJ-6 cells treated with mAb16D10 or not treated. Relative to non-transfected cells, a significantly decreased percentage of fluorescent transfected cells, reflecting caspase cleavages triggered by mAb16D10 treatment, was noted with the two p53-targeted siRNAs (Fig. 4C). Again, siRNA 2 was more efficient than siRNA 1. We concluded that p53 was thus functional and required for caspase activation.

The ability of p53 to promote cell death can be linked to its ability to induce Bax transcription directly (27) and to alter that of Bcl-2 or its activity (28). Consistent with this, we found that both the expression of proapoptotic Bax protein and inhibition of expression of antiapoptotic Bcl-2 protein resulted from cell mAb16D10 treatment but not from irrelevant Ab or medium treatment (Fig. 4D). Bax/Bcl-2 expression was not modified in mAb16D10-treated PANC-1 cells compared with controls. Hence, functional p53, Bax, and caspases 9, 3, and 7 play essential roles in the programmed death of tumor cells expressing 16D10 Ag.

**mAb16D10 promotes GSK-3β activation**

Cell treatment with mAb16D10 caused a G1/S arrest (G1/S peak: 96 versus 71% for control) (Fig. 5A, dashed area) and an increase of dead cells (sub-G1 peak: 6 versus 0.8%) (Fig. 5A, blue area), whereas cells in G2/M phase decreased (G2/M peak: 4 versus 29%) (Fig. 5A, red area). These results were confirmed with cells synchronized in G1/S phase by aphidicolin. To note, mAb16D10 treatment had no effect on the cell cycle of PANC-1 cells (data not shown).

Next, we examined the status of GSK-3β in mAb16D10-treated SOJ-6 cells. This kinase affects expression of cyclin D1, a modulator of the cell cycle. Whereas cell treatment did not affect the expression of total GSK-3β, the Ser9-phosphorylated-GSK-3β (inactive) form was decreased, contrary to controls (Fig. 5B). Fig. 5C shows that cell treatment decreased cyclin D1 expression, inversely to GSK-3β activation.

A dramatic amount of phosphorylated β-catenin, which is phosphorylated by GSK-3β, was detected in SOJ-6 cells treated with mAb16D10, contrary to controls (Fig. 5C). To get insight into the GSK-3β functional implication, GSK-3β activity was inhibited with lithium chloride. This inhibition reversed the effects triggered by mAb16D10 in SOJ-6 cells: we observed strongly decreased phos-
phorylation of β-catenin (Fig. 5D), inhibition of p53 expression (Fig. 5E), and a significantly decreased percentage of fluorescent cells with caspase cleavage (Fig. 5F). Notably, our data evidenced functional relationships between GSK-3β and both β-catenin and p53.

**mAb16D10 alters β-catenin localization and E-cadherin expression**

Next, we sought to determine whether treatment with mAb16D10 could alter β-catenin localization together with phosphorylation status. After treatment with mAb16D10, β-catenin was detected mainly in the cytosolic compartment of SOJ-6 cells, under phosphorylated form, whereas total β-catenin was localized at the plasma membrane of untreated cells (Fig. 6A).

SOJ-6 cells constitutively do not exhibit detectable plasma membrane E-cadherin. Upon mAb16D10 treatment, SOJ-6 cells markedly expressed E-cadherin at the plasma membrane (Fig. 6B). The ganglioside GM1, which is selectively partitioned in lipid microdomains, colocalized with E-cadherin in mAb16D10-treated SOJ-6 cells.
SOJ-6 cells (Fig. 6C). E-cadherin expression was confirmed by immunoblots of cell lysates of SOJ-6 cells treated with mAb16D10 but not in lysates of irrelevant Ab- or mock-treated cells (Fig. 6D). PANC-1 cells, which did not exhibit membrane-16D10 Ag (Fig. 1B), did not express E-cadherin at a detectable level at their plasma membrane, with or without mAb16D10 treatment (Fig. 6B). Thus, E-cadherin expression at the plasma cell membrane depended on both the presence of the 16D10 Ag at the cell surface and its ligation to mAb16D10.

Disorganization of membrane lipid microdomains decreases the antiproliferative effects of mAb16D10

Fig. 7A shows the 16D10 Ag colocalized with GM1 at the SOJ-6 cell surface. To address the role of lipid microdomains, we used MβCD and filipin, drugs reported to deplete cholesterol in membrane rafts or to sequester cholesterol, respectively, without altering cell viability (29). In the presence of MβCD and filipin, the effects of mAb16D10 on the cell viability significantly decreased (Fig. 7B). Furthermore, MβCD decreased the expression of the 16D10 Ag contrary to filipin (Table I; Supplemental Fig. 1).

Because sphingolipids also participate in the raft structure, we used metabolic inhibitors of (glyco)sphingolipid biosynthesis (15). Tested at efficient concentrations, neither L-cycloserine (an inhibitor of serine palmitoyltransferase) nor fumonisin 1 or 2 (inhibitors of dihydroceramide synthetase) interfered with the antiproliferative effects of mAb16D10 (Fig. 7C). We next tested PDMP, an inhibitor of glycosphingolipid synthesis, acting on the last step of sphingolipid synthesis (30). PDMP partly reversed the effects of mAb16D10 on SOJ-6 cell proliferation. The interaction between mAb16D10 and 16D10 Ag on pancreatic tumor cells at the level of membrane lipid microdomains is illustrated in Fig. 8.

Discussion

Resistance of pancreatic cancer to apoptotic cell death is a major factor preventing responses to current therapies. In this study, we show that mAb16D10 is able to induce pancreatic tumoral cell death. The decrease of cell viability induced by mAb16D10 requires 16D10 Ag expression at the tumor cell surface, as human PANC-1 cells, which do not express this Ag, are insensitive to mAb16D10, contrary to other tumor cell lines that express 16D10 Ag, such as SOJ-6 and BxPC-3. Moreover, cell cycle analysis indicates that tumor cells treated with mAb16D10 or irrelevant IgM for 24 h. Bcl-2 and Bax expressions by SOJ-6 and PANC-1 tumor cells were probed with specific Abs. β-Actin was used as internal control. Representative immunoblot of three independent experiments.

SOJ-6 cells (Fig. 6C). E-cadherin expression was confirmed by immunoblots of cell lysates of SOJ-6 cells treated with mAb16D10 but not in lysates of irrelevant Ab- or mock-treated cells (Fig. 6D). PANC-1 cells, which did not exhibit membrane-16D10 Ag (Fig. 1B), did not express E-cadherin at a detectable level at their plasma membrane, with or without mAb16D10 treatment (Fig. 6B). Thus, E-cadherin expression at the plasma cell membrane depended on both the presence of the 16D10 Ag at the cell surface and its ligation to mAb16D10.

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FIGURE 5. mAb16D10 arrests cell cycle in G1/S phase and promotes GSK-3β activation in SOJ-6 cells. (A) Cell cycle distribution of SOJ-6 cells synchronized or not with aphidicolin (5 μg/ml) for 6 h, further incubated with or without mAb16D10 for 24 h. Each experiment was carried out in triplicate. (B and C) SOJ-6 cells were treated for 24 h with mAb16D10 or irrelevant IgM. Total GSK-3β, phospho-GSK-3β, cyclin D1, total β-catenin, and phospho-β-catenin expressions were detected in cell lysate by Western blot using specific Abs. β-Actin was used as internal control (50 μg total protein per lane). Representative immunoblot of three independent experiments. (D and E) SOJ-6 cells were treated with mAb16D10 or irrelevant IgM for 24 h after 1-h preincubation with 5 mM lithium chloride (LiCl), an inhibitor of GSK-3β activity. Representative immunoblot of p53 from whole-cell lysates showing the effects of LiCl (D) on β-catenin, (E) on p53 expression. β-Actin was used as internal control. (F) Functional effects of mAb16D10 on LiCl-treated cells monitored by CaspACE FITC-VAD-FMK In Situ Marker. Maximal number of fluorescent cells was taken as 100%. Results are mean ± SD of three independent experiments. ***p < 0.001.
directly activate the proapoptotic Bax in the cytosol to permeabilize mitochondria membrane (33), and simultaneously downregulates Bcl-2 (27). These events result in caspase activation and inactivation of PARP1, which plays an essential role in repair of damaged DNA (34), and finally trigger cell death (27, 33). The gene TP53 has a common sequence polymorphism in the proline-

FIGURE 6. mAb16D10 alters β-catenin localization and phosphorylation and E-cadherin expression at the plasma membrane of tumor cells expressing 16D10 Ag. (A) SOJ-6 cells treated with mAb16D10 were labeled with Abs to total β-catenin and phospho–β-catenin before fluorescence microscope analyses (original magnification ×400). (B) After treatment with mAb16D10, SOJ-6 and PANC-1 cells were labeled with Ab to E-cadherin (original magnification ×400). (C) SOJ-6 cells treated with mAb16D10 were probed with Ab to E-cadherin; ganglioside GM1 was stained with Alexa 488–cholera toxin subunit B (CTB) and cross-linked by anti-CTB according to the manufacturer’s instructions before confocal microscopy analyses (original magnification ×945). (D) SOJ-6 cells were treated with mAb16D10 or irrelevant IgM for 24 h, and cell lysate proteins were analyzed by Western blot using Abs to E-cadherin. β-Actin was used as internal control (50 μg total protein per lane).

FIGURE 7. Ag 16D10 is localized in membrane lipid microdomains, and disorganization of these microdomains decreases the antiproliferative effects of mAb16D10. (A) SOJ-6 cells were probed with mAb16D10; ganglioside GM1 was stained with Alexa 488–CTB and cross-linked by anti-CTB before confocal microscopy analyses (original magnification ×945). (B and C) The culture medium of SOJ-6 cells was replaced by fresh medium containing MJSCD or filipin (B) or metabolic inhibitors of glycosphingolipid biosynthesis at indicated concentration for 6 h (C). Next, the medium was replaced with fresh medium containing drugs plus mAb16D10 or irrelevant Abs for 24 h. The amount of living cells was determined by monitoring their mitochondrial respiratory chain activity using MTT assay. *p < 0.05, **p < 0.001 (versus control). Results are expressed as a percentage relative to controls without drugs; data are means ± SD of three independent experiments. F1, Fumonisin 1; F2, fumonisin 2; LCS, t-cycloserine.
Table I. Cell surface 16D10 Ag expression analyzed by flow cytometry using mAb16D10 on SOJ-6 cells treated with MJ1CD and filipin

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<th>MJ1CD (µM)</th>
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\(^a\)Mean fluorescence intensity of cells stained with mAb16D10.
\(^b\)Percentage of variation versus control; average of three independent experiments.

rich domain of p53, which is necessary for the protein fully to induce apoptotic cell death (25). This results in either proline or arginine at amino acid position 72, the latter inducing apoptosis markedly better than the Pro72 variant. Notably, the p53 variant expressed by SOJ-6 cells contains Arg72. It also contains one mutation of Tyr236 to Asp236, which may affect the protein dynamic (35). Importantly, silencing of p53 expression in SOJ-6 cells significantly decreases cell death induced by mAb16D10, which substantiates the notion that mAb16D10 evokes cell death by activation of the p53-dependent apoptotic pathway.

Besides, activation of p53 leads to diverse cellular responses, including cell cycle arrest involving effector proteins, in particular p21 for G1 arrest (23, 36), and thus might be involved in the cell cycle arrest in the G1/S phase of mAb16D10-treated SOJ6 cells. Also, treatment of tumor cells with mAb16D10 favors the activation of GSK-3\(^b\), which substantially phosphorylates \(\beta\)-catenin, associated with a markedly decreased cyclin D1 expression. Cyclin D1, whose overexpression is most frequently associated with human cancer (30), shortens the G1/S phase transition and promotes cell progression and differentiation (37). In this, decreased cyclin D1 expression may rely on nonexclusive mechanisms. Others reported that activated GSK-3\(^b\) phosphorylates \(\beta\)-catenin in the cytoplasm; once phosphorylated, it is degraded and unable to participate in regulation of transcription of target genes like cyclin D1 (28-40). Besides, activated GSK-3\(^b\) translocates into the nucleus and phosphorylates cyclin D1, which is afterward exported from the nucleus and degraded by the ubiquitin-dependent proteasome complex (37, 38). Our data demonstrate that after exposure to an inhibitor of GSK-3\(^b\) activity, mAb16D10-treated SOJ-6 cells no longer express \(\beta\)-catenin under phosphorylated form. Consistent with the above studies, \(\beta\)-catenin therefore should be available to intervene in the regulation of transcription of cyclin D1 gene.

GSK-3\(^b\) has direct or indirect relationships with p53. Once activated, GSK-3\(^b\) indirectly may negatively regulate p53 through phosphorylation and activation of Mdm2, an important negative regulator of p53 (41). However, after DNA damage, GSK-3\(^b\) may positively and directly interact with p53, in both nucleus and mitochondria, contributing to the transcriptional and apoptotic actions of p53 (42). Therefore, we investigated the functional interaction between transcriptional expression of p53 and GSK-3\(^b\) activation induced via the binding of the Ab to its cognate Ag. Two arguments suggest that p53-mediated caspase activation involves GSK-3\(^b\). The inhibition of GSK-3\(^b\) activity by LiCl first markedly inhibited p53 expression suggesting that GSK-3\(^b\) activity and mAb16D10-induced p53 expression are linked. Second, it also led to dramatically decreased cell death. Together, these results may imply that GSK-3\(^b\) positively regulates p53 level and promotes its action.

GSK-3\(^b\) also regulates the interaction of \(\beta\)-catenin with E-cadherin (43). Serine residues in the E-cadherin cytoplasmic domain are phosphorylated by GSK-3\(^b\) and casein kinase II, creating additional interactions with \(\beta\)-catenin. In this study, we demonstrated that mAb16D10 treatment induces plasma membrane E-cadherin expression in SOJ-6 cells, whereas intriguingly, \(\beta\)-cat-
enin is mainly detected in the cytosolic compartment, in contrast to untreated SOJ-6 cells. Thus, β-catenin no longer seems to bind to E-cadherin and is translocated from the plasma membrane to the cytoplasm in mAb16D10-treated tumor cells. Moreover, the increased expression of phospho-β-catenin upon mAb16D10 cell treatment and its cytoplasmic translocation suggest further degradation via the proteasome (39, 44).

The E-cadherin/β-catenin-mediated cell adhesion system is known to act as a suppressive system for invasion (45). When β-catenin is bound to E-cadherin, signaling-competent nuclear β-catenin levels diminish, and cell proliferation and invasion are suppressed (45). E-cadherin/β-catenin expression is lost in invasive digestive cancers (46, 47); partial or complete loss of E-cadherin expression correlates with malignancy (48), whereas overexpression of E-cadherin leads to defective invasion of melanoma cells (49). Thus, alteration in cadherin/catenin function or expression is found in the neoplastic process as a step in metastasis (4).

Our data are in line with the results of Leong’s et al. (50) showing that reexpression of E-cadherin in E-cadherin-negative breast tumors (due to inhibition of ligand-induced Notch signaling) is associated with suppression of active β-catenin, with apoptosis and with both tumor growth and metastasis inhibition. Notably, chronic exposure of A431 epidermoid carcinoma to cetuximab induces an upregulation of E-cadherin expression (51). Because morbidity in most cancer patients is not due to primary cancer but to metastatic disease, which is particularly true in PDAC (1), the high expression of E-cadherin promoted by cell treatment with mAb16D10 would be beneficial to patients by leading to defective malignancy of pancreatic tumor cells.

16D10 Ag and E-cadherin, associated with β-catenin, are confined in lipid microdomains, in agreement with the finding that BSDL is associated with intracellular rafts during transportation to cell membrane (15). Notably, mAb16D10 binding seems to require a cholesterol-dependent intact structure of lipid microdomains to trigger cell death as both depletion in cholesterol and/or or Ag and sequestration of cholesterol associated with a marked Ag expression lead to reversion of antiproliferative effects of mAb1610. The association of 16D10 Ag with membrane lipid microdomains occurs once these domains are structured because early inhibitors of sphingolipid synthesis are inefficient in reversing mAb16D10 effects. This inefficiency also suggests that the salvage pathway recycling surface glycosphingolipids (52) is sufficient to ensure the formation of lipid rafts to which the 16D10 Ag associates. Only PDMP, which blocks ganglioside synthesis (53), partially decreased cell death induced by mAb16D10. This might be because the inhibitor impairs the intracellular pathway of BSDL (15) and/or that the drug affects the association of the 16D10 Ag-bearing protein with lipid rafts, as for the ErbB2 Ag (54). Thus, the integrity of cholesterol-rich lipid microdomains might be a prerequisite to the presence of the 16D10 Ag at the surface of pancreatic tumor cells.

Rozibak et al. (55) showed that β-catenin and E-cadherin complexes are associated with the polycystins and the raft marker flotillin-2 in primary human kidney epithelial cells. These molecules might confer to rafts the role of signaling platforms. This notion is supported by substantial evidence that raft integrity is crucial for the initiation and maintenance of intracellular signals (56, 57). Thus, E-cadherin, associated with β-catenin, and 16D10 Ag in lipid rafts might therefore belong to signaling platforms involved in the induction of cell cycle arrest and cell death. At present, we do not know the exact relationships between tumor Ag and the complex E-cadherin–β-catenin and signal transductions involving p53 and GSK-3β. The prime mover may be that binding of mAb16D10 to tumor Ag at the cell surface triggers destabilization of β-catenin and E-cadherin complexes favoring the phosphorylation of β-catenin by GSK-3β. This requires further thorough investigations in such peculiar cells that are tumor cells. To summarize, mAb16D10 exerts pleiotropic and converging effects on pancreatic cells expressing the 16D10 Ag to trigger cell death, illustrated in Fig. 8.

Inducing apoptosis of pancreatic cancer cells by targeting protein is a major goal for development of therapeutic agents. mAb16D10, which specifically recognizes tumor cell surface Ag of human pancreatic tissues (16) and inhibits the development of xenografted human pancreatic tumor (17), causes death of human pancreatic tumor cells (this study). These data strongly suggest mAb16D10 may fight against PDAC. Moreover, the high expression of E-cadherin promoted by cell treatment with mAb16D10 would be beneficial to patients by leading to defective malignancy of pancreatic tumor cells. Therefore, mAb16D10 represents a promising tool in the development of new Ab-based therapeutic strategies, using chimeric- or humanized-Ab versions, against pancreatic adenocarcinoma, hopefully reinforced by the immune system through recognition of apoptotic tumor cells.

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Disclosures

The authors have no financial conflicts of interest.

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