Review

Cell Adhesion Is a Key Determinant in de Novo Multidrug Resistance (MDR): New Targets for the Prevention of Acquired MDR

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Abstract
Clinical circumvention of multidrug resistance (MDR) is a Sisyphean task faced in the treatment of many cancers. Identification of several mechanisms of acquired MDR has led to the development of chemosensitizing agents that counter specific mechanisms of MDR. Initial successes in therapy using "chemosensitizers" often culminate in relapse due to the multifactorial nature of acquired MDR. Therefore, it may be important to design therapeutic strategies that focus on mechanisms that allow for cell survival after initial treatments, before the acquisition of MDR. It has been proposed that extracellular effectors such as cytokines, matrix components, and adjacent cells may provide sanctuary to cancer cells by preventing stress-induced cell death. This review focuses on research implicating the cancer cell environment as a particularly important determinant in the emergence of drug resistance. More specifically, we will discuss the role of direct contact between cancer cells and the extracellular matrix or with adjacent cells as extrinsic effectors of de novo MDR. Cell adhesion has been demonstrated to prevent cell death through a number of mechanisms. Identification of cell adhesion-mediated drug resistance as an initial de novo effector of MDR suggests that therapies targeting interactions between cancer cells and their environment may lead to the sensitization of cancer cells to chemotherapy or radiotherapy before the emergence of acquired mechanisms of MDR.

Introduction
In the genetic background of cancer, an increasing body of evidence indicates that the tumor microenvironment may also contribute to cancer progression. Reports demonstrate that soluble factors such as cytokines, hormones, and growth factors (1–4), as well as interactions between tumor cells and ECM2 molecules (5, 6) or adjacent cells (7, 8), may play a significant role in the pathogenesis and progression of human cancers. Furthermore, recent studies suggest that these same environmental factors may also contribute to the survival of cancer cells after initial therapy, allowing resistant cells to proliferate and acquire multiple mechanisms of drug resistance (Table 1). Identifying mechanisms associated with this environmental influence on treatment response may facilitate the development of therapeutic approaches to prevent acquired mechanisms of MDR.

Chemotherapy and radiotherapy remain the standard treatment of both hematopoietic cancers and solid tumors. Many cancers are characterized by an initial sensitivity to chemotherapy; however, acquired resistance to therapy invariably leads to patient relapse through the expansion of a multidrug-resistant population of cancer cells. This phenomenon of acquired MDR spawned a large effort to identify molecular targets of cytotoxic agents and the mechanisms of cellular resistance, with the goal of developing strategies to overcome MDR. Examination of in vitro drug-selected cell lines led to the identification of four classes of acquired MDR: (a) reduced drug accumulation; (b) alterations in drug target; (c) increased repair of drug-induced damage; and (d) inhibition of apoptotic signaling pathways (Fig. 1). These classical mechanisms of acquired MDR are primarily intrinsic or genetic in nature and have been elucidated using cellular systems that focus exclusively on molecular changes within individual cells under prolonged exposure to cytotoxic insult. These in vitro models have provided an invaluable tool for the identification of mechanisms of acquired MDR. However, one disadvantage of these models is that they do not take into consideration the effects of the tumor microenvironment. The observation of acquired drug resistance, taken together with the clinical observation that acquired mechanisms of MDR develop only after prolonged treatment, suggests that in vivo there may be initial antiapoptotic mechanisms that promote cell survival. We propose that unique extracellular environments may mediate these initial or de novo resistance mechanisms and, in turn, facilitate the emergence of classical mechanisms of MDR.

In this review, we discuss the role of environmental factors in MDR, focusing primarily on the effects of adhesion to the microenvironment.

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2 The abbreviations used are: ECM, extracellular matrix; MDR, multidrug resistance; CAM-DR, cell adhesion-mediated drug resistance; MM, multiple myeloma; P-gp, P-glycoprotein; topo, topoisomerase; MGMT, O6-methylguanine DNA methyltransferase; FN, fibronectin; 4-HC, 4-hydroxy-cyclophosphamide.

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The mechanisms of resistance identified in these in vitro studies fall into four basic categories. (a) Reduced intracellular drug accumulation has historically been associated with overexpression of the ATP-binding cassette transporter, P-gp/MDR-1 (22) and related drug transporters, including MDR protein 1 (23), and breast cancer resistance protein (24). This family of proteins is characterized by reduced drug accumulation at the target involving the transport of natural product agents, including vincristine, doxorubicin, and Taxol, and others (25). Additionally, the major vault protein, lung resistance protein, has also been associated with drug resistance via a redistribution of doxorubicin from the nucleus to cytoplasm without overall changes in total cellular drug accumulation (26). (b) Alteration in drug targets is another category of acquired drug resistance mechanisms. This mechanism of MDR is best characterized by alterations in the expression and function of DNA topoisomerases. topo II is an ATP-dependent enzyme that functions during DNA replication, RNA transcription, and chromosomal condensation and separation to release torsional stress on DNA by cleaving and passing both strands of DNA through the topo II protein-DNA complex (27). topo II family members (topo IIa and topo IIb) are targets for several classes of chemotherapeutic drugs, including anthracylines (doxorubicin), anthracyclines (mitoxantrone), and epipodophyllotoxins (etoposide). The genotoxic nature of these compounds manifests from the stabilization of DNA-topo II complexes after DNA cleavage, resulting in the accumulation of DNA double-strand breaks (27). Resistance to these topo II inhibitors has been shown to result from decreased levels of topo II expression and decreased enzy-

### Classical Mechanisms of Acquired MDR

The emergence of acquired MDR is a major hurdle in the successful treatment of cancer. MM has been an ideal model for examining the emergence of MDR. MM is a considered a drug-sensitive disease because patients are characterized by an initial response to chemotherapy but invariably relapse after the development of MDR in a population of cells. Studies examining the acquisition of MDR in vitro have led to the identification of several potential molecular targets for chemotherapy. In this review, we will discuss just a few well-documented players in acquired MDR to provide examples of the general characteristics of mechanisms of acquired MDR (for a more complete review of acquired MDR, see Refs. 18–21).

<table>
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<tr>
<th>Cytotoxic stress</th>
<th>Protective factor</th>
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<tr>
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<tr>
<td>Etoposide</td>
<td>FN/α5, β1, and β3</td>
<td>Murine TDECs</td>
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<td>Cyclophosphamide</td>
<td>Cell-cell contact (spheroid model)</td>
<td>EMT-6 mammary carcinoma</td>
<td>8</td>
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<tr>
<td>Doxorubicin</td>
<td>FN/α5β1 and α3β1</td>
<td>RPMI 8226</td>
<td>11</td>
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<tr>
<td>Melphalan</td>
<td>FN, LN, Col. IV, and TN/β1</td>
<td>SCLC</td>
<td>12</td>
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<td>RPMI 8226</td>
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*Col., collagen; CML, chronic myelogenous leukemia; TDEC, tumor-derived endothelial cells; TN, tenascin.*

ECM components and adjacent cells on sensitivity to cytotoxic agents. Identification of CAM-DR (8–16) indicates that specific interactions between cancer cells and their environment may be important targets for novel treatments of hematopoietic and solid cancers. As we will discuss, several mechanisms of CAM-DR have been identified (8, 15–17). We have classified these mechanisms of de novo drug resistance under four general categories: (a) decreased cellular proliferation; (b) alterations in drug target; (c) decreased apoptosis; and (d) integrin signaling cascades and cytoskeletal rearrangements (Fig. 1). Importantly, the de novo nature of CAM-DR suggests that we may be able to target early effectors of cell survival that prevent treatment-induced apoptosis. Doing so should enhance the efficacy of initial cancer treatment and prevent the acquisition of MDR.
matic activity resulting from mutations in specific domains of topo II (28, 29). (c) Many classes of chemotherapeutic drugs elicit their cytotoxic effects by damaging DNA. A likely mechanism of MDR is increased rates or levels of DNA repair. For example, the DNA repair enzyme MGMT catalyzes the removal of methyl adducts of the O\textsubscript{6} of guanine resulting from treatment with nitrosoureas (30). Levels of MGMT enzymatic activity have been shown to correlate with sensitivity to nitrosourea-mediated cell death (31), suggesting that MGMT may be an important effector of drug resistance. (d) Evidence has accumulated to support the hypothesis that chemotherapeutic drugs use physiological apoptotic pathways to mediate cell death and are therefore susceptible to endogenous inhibitors of apoptotic signaling (32–37). The Bcl-2 family of proteins has been demonstrated to play a major role in the regulation of programmed cell death (38) and has recently been shown to correlate with acquired MDR (39–41). Reports also suggest that MDR may result from other alterations in apoptotic signaling. Studies supporting the involvement of physiological mediators in cell death mediated by certain drugs have focused on the CD95/CD95 ligand pathway (35, 36). However, a direct contribution of the CD95/CD95 ligand interaction to drug-induced cell death remains controversial, and in most cell systems, the common mediators of cell death are distal to the death receptor-ligand interaction (32–34, 42).

The identification of mechanisms of acquired drug resistance facilitated research into the identification of specific drug targets and the development of strategies to sensitize multidrug-resistant cells to therapy. For instance, chemosensitizers, a class of compounds including verapamil, cyclosporine A, and its derivative, PSC 833 (Novartis Pharmaceutical, East Hanover, NJ), block the drug export protein P-gp (43). However, data thus far suggest that chemosensitization therapies are characterized by an acquisition of chemosensitizer-insensitive mechanisms of MDR due to the multifactorial nature of acquired MDR (43–48). In support of this, Futscher et al. (44) demonstrated that culture of 8226 MM cells in the presence of doxorubicin or of doxorubicin and the chemosensitizer verapamil selected for distinct mechanisms of acquired drug resistance. Selection with doxorubicin alone resulted in increased P-gp expression. In contrast, concomitant selection with doxorubicin and verapamil resulted in decreased levels of topo II protein and activity, with no increase in P-gp expression. Because the MDR phenotype is multifactorial, cancer cells were able to overcome the initial effects of chemosensitizers on one mechanism of MDR (reduced drug accumulation; increased P-gp expression) by enlisting other mechanisms (altered drug target; decreased topo II levels and activity) to combat drug-induced cytotoxicity (44). The multifactorial nature of MDR indicates that it may be important to target the antiapoptotic processes that...
prevent initial drug-induced cytotoxicity and facilitate the emergence of acquired drug resistance mechanisms. To accomplish this, we need to characterize these initial or de novo mechanisms of resistance and determine how they influence the acquisition of MDR.

More than a decade ago Teicher et al. (49) established that drug selection in vivo differed from selection in vitro. Teicher et al. (49) showed that EMT-6 murine mammary tumors selected for resistance to cis-diamminedichloroplatinum, carboplatin, cyclophosphamide, or thiotapec in vivo for 6 months lost the drug-resistant phenotype when cultured in vitro. Moreover, the authors observed a loss of drug resistance when cells were passed in vivo in the absence of cytotoxic agents (except thiotapec). These results suggest that the cellular environment plays a significant role in selection for in vivo drug resistance, possibly in response to the cytotoxicity of chemotherapy. In support of these findings, we later demonstrated that drug selection in myeloma cell lines correlated with increased adherent potential. (11, 13) Damiano et al. (11) initially demonstrated that \( \beta_1 \) integrin-mediated adhesion of the RPMI 8226 MM cell line to FN blocked cell death induced by doxorubicin and melphalan as compared with cells maintained in suspension. Furthermore, comparison of the parental, drug-sensitive 8226 cell line with 8226/Dox6 and 8226/LRS drug-selected cell lines (doxorubicin- and melphalan-selected cell lines, respectively) demonstrated a significant increase in \( \alpha_5\beta_1 \) integrin expression and FN adhesion in the drug-resistant variants. Culture of the drug-resistant cell lines without selective pressure (doxorubicin or doxorubicin and melphalan) resulted in a reversion in sensitivity to cytotoxic insult and decreased \( \alpha_5\beta_1 \) expression and adhesion. These findings suggest that drug resistance is concomitant with increased adherent potential. In a subsequent study, Damiano and Dalton (13) demonstrated that melphalan-selected drug resistance correlated with a 5-fold increase in \( \alpha_5\beta_1 \)-specific adhesion to FN over the parental drug-sensitive U266 MM cell line without detectable changes in \( \alpha_5\beta_1 \) expression. This observation demonstrated a correlation between melphalan drug selection in the U266 cell line and a change from a low to high affinity state of \( \alpha_5\beta_1 \), possibly involving inside-out activation of integrin receptors. Together, these reports allowed us to speculate that this integrin-primed status, resulting from either increased expression of \( \alpha_5\beta_1 \) or increased ligand affinity, may establish an antiapoptotic state that participates in the selection process. These observations suggest that in vivo interactions between tumor cells and the microenvironment may be involved in the early stages of drug selection, providing an initial survival advantage to FN-adherent cells, and promote the acquisition of the classical forms of drug resistance.

**Antia apoptotic Effects of Adhesion**

Studies demonstrating that detachment of anchorage-dependent cells resulted in apoptosis or anokis were the first to show that integrin-mediated adhesion directly participated in cell survival (24, 50). These initial works primarily involved anchorage-dependent “normal” endothelial and epithelial cell models (24, 50). It was hypothesized that matrix-independent growth was (is) a major step in cellular transformation leading to cancer because transformed cells lose the requirement for adhesion (50). However, we propose that cancer cells also use the antiapoptotic effects of matrix adhesion for survival. Adhesion of hematopoietic and solid cancer-derived cells to FN has been shown to promote ex vivo growth (5, 51, 52) and protection from serum deprivation (6, 53), suggesting that, as in normal anchorage-dependent cells, matrix-cell interactions contribute to regulation of antiapoptotic events in cancer cells. More recently, reports indicate that cancer cells also use these antiapoptotic signals to evade chemotherapy and radiotherapy (Table 1; Ref. 54).

**Integrin Adhesion and Activation**

Integrin ligation has been demonstrated to protect both hematological and solid tumor cells from a number of apoptotic stimuli. Integrins are a family of 18 \( \alpha \) and 8 \( \beta \) transmembrane receptors that combine to form heterodimeric receptors (55, 56). “Outside-in” signaling from integrins is characterized by the formation of focal adhesions that integrate environmental signals with intracellular responses, such as cytoskeletal rearrangements and signal cascades controlling cell migration, proliferation, and survival (57–59). Integrin-matrix interactions are regulated through a multistep process involving either alterations in integrin surface expression or alterations in ligand affinity (60–62). Integrin ligand affinity can be positively or negatively regulated by intracellular or “inside-out” signaling that stimulates or represses the activation state of \( \alpha \) \( \beta \) heterodimers (55, 61, 63). Inside-out integrin ligand affinity is regulated by a number of signaling factors including the small GTPases, Ha-Ras, R-Ras, and Rap1 (61, 64–67). Zhang et al. (61) showed that expression of a constitutively active R-Ras in murine and human hematopoietic cell lines became highly adherent to FN, as compared with cells transfected with wild-type R-Ras or vector alone. No detectable alterations in \( \alpha_5 \), \( \alpha_4 \), \( \alpha_5 \), or \( \beta_1 \) surface expression were observed, indicating that R-Ras activity facilitated increased integrin ligand affinity. Others have demonstrated that expression of Ha-Ras facilitated high affinity integrin binding through a pathway independent of R-Ras in certain cell systems (64, 65). Inside-out regulation of leukocyte integrin ligand avidity has been shown to involve the small G protein Rap1 (67, 68). These studies demonstrate that the prenylated G proteins, Ha-Ras, R-Ras, and Rap1, play a significant role in cellular adhesion by regulating the inside-out activation of integrins.

**CAM-DR**

It is well established that hematopoietic and solid cancer cells express cell adhesion molecules that participate in cellular dissemination and metastasis. This fact, taken together with the growing number of studies describing the prosurvival effects of integrin adhesion, has led us to pose the following question: do these adhesion molecules (integrins) expressed on cancer cells participate in disease progression by conferring a survival advantage to adherent cancer cells? To address this question, our laboratory examined the effects of adhesion to FN, a prominent ECM component of the
bone marrow (38), on cellular response to physiological and therapeutic cytotoxic insult on MM cell lines. FN associates with the integrin receptors very late antigen-4 (α9β4) and very late antigen-5 (α6β1) and has been demonstrated to mediate prosurvival effects in several cell systems (54). In Table 1, we summarize findings demonstrating that cellular adhesion to ECM components as well as to adjacent cells confers a protective advantage to both hematopoietic and solid tumor models in response to chemotherapeutic and physiological mediators of apoptosis.

As illustrated in Table 1, cell adhesion has been observed to protect cells from an array of cytotoxic agents. The multidrug-resistant nature of this phenomenon suggests that cellular adhesion may act through several drug-specific mechanisms or through an effector common to several categories of cytotoxic agents, possibly involving direct control of the apoptotic cascade. We have shown that CAM-DR in RPMI 8226 MM cells is associated with an inhibition of mitoxantrone-induced caspase-3 and caspase-7 cleavage (Fig. 2), suggesting that the mechanism of CAM-DR may involve a direct inhibition of a point(s) in the apoptotic signal cascade. However, as discussed below, cell adhesion-mediated MDR may occur through a number of mechanisms.

**Reduced Cellular Proliferation.** Delineation of the individual molecular mechanisms involved in acquired drug resistance may lead to the development of a number of targets for reversal of MDR. Investigation into the mechanisms of CAM-DR may similarly identify specific regulatory determinants that lend themselves to therapeutic modulation. In the last 30 years, adhesion-regulated events have been demonstrated to participate in CAM-DR in both hematopoietic and solid tumor models. Sutherland and Durand (7) demonstrated in an in vitro solid tumor model that intercellular contact in spheroid culture regulated sensitivity to radiation exposure. In a similar model system, St. Croix et al. (8) showed that spheroid culture of EMT-6 mammary tumor cells inhibited 4-HC- and γ-irradiation-mediated cell death when compared with cells cultured as a monolayer. This intercellular adhesion-mediated resistance correlated with a 15-fold increase in the expression of cyclin-dependent kinase p27Kip1 and decreased proliferation. Treatment of spheroid cells with p27 Kip1 antisense but not mismatch oligonucleotides considerably reduced the tumorigenicity of 4-HC-treated tumor cells, indicating that p27Kip1 plays a critical role in intracellular contact-mediated resistance to 4-HC. In a subsequent report, St. Croix et al. (69) demonstrated that E-cadherin-mediated intracellular adhesion increased p27Kip1 levels, suggesting that E-cadherin binding was important for growth-suppressive effects of intracellular adhesion. However, no direct link was established between E-cadherin-mediated adhesion and CAM-DR. Our laboratory recently demonstrated that adhesion to immobilized FN provided a survival advantage to cell death induced by a panel of cytotoxic agents in hematopoietic cancer cell lines (11, 13–16). Hazlehurst et al. (15) has since demonstrated that prolonged adhesion (48 h) of RPMI 8226 cells to FN resulted in a reversible reduction in cell proliferation, a concomitant accumulation of cells in the G1 phase of the cell cycle, and resistance to etoposide. FN adhesion coincided with a reversible increase in protein levels of p27Kip1. Treatment of FN-adherent cells with p27Kip1 antisense oligonucleotides reversed CAM-DR with no effect on adhesion. These results
show a direct link between p27\(^{Kip1}\) expression, cytoprotection, and \(\beta_i\) integrin-mediated adhesion. Together, these studies demonstrate that the cell cycle-regulatory protein p27\(^{Kip1}\) plays an important role in CAM-DR in both solid and hematopoietic tumor cells. In both reports, increased p27\(^{Kip1}\) protein levels corresponded with resistance to cytotoxic insult and cell cycle arrest, demonstrating a link between reduced cell proliferation, reduced sensitivity to apoptotic stimuli, and cellular adhesion. However, Hazlehurst et al. (15) demonstrated that p27\(^{Kip1}\) levels increased within 2 h of adhesion to FN, and previous results demonstrated that the CAM-DR phenotype is observed as early as 2 h after adhesion of 8226 cells to FN (13). The discrepancy between time of cell cycle arrest, up-regulation of p27\(^{Kip1}\), and the emergence of CAM-DR suggests a potential divergence in the cell cycle and antiapoptotic effects of p27\(^{Kip1}\). Recent reports have shown that p27\(^{Kip1}\) may have specific antiapoptotic functions independent of its historical role in proliferation (70, 71). Eymin et al. (70, 71) demonstrated that p27\(^{Kip1}\) is a direct substrate for caspases and that expression of cDNA encoding p27\(^{Kip1}\) inhibited etoposide-induced apoptosis. Taken together with the results published by St. Croix et al. (69) and Hazlehurst et al. (15) demonstrating increased levels of p27\(^{Kip1}\) after cellular adhesion, this suggests that in addition to its cell cycle-regulatory functions, p27\(^{Kip1}\) may also directly affect programmed cell death. Therefore, the antiapoptotic effects of p27\(^{Kip1}\) after adhesion may occur through a direct action on apoptotic signaling.

**Alterations in Drug Target.** Another mechanism by which CAM-DR has been shown to reduce drug cytotoxicity involves alterations in drug target. However, unlike the acquired mechanisms affecting drug target topo II that involve decreased expression or mutations in essential domains (28, 29), adhesion-mediated alterations involve changes in the subcellular localization of the drug targets. Oloumi et al. (17) showed in spheroid culture of a number of solid tumor cell models that the outer rim cells of spheroids were resistant to etoposide when compared with cells grown on a monolayer. The resistance observed correlated with a redistribution of topo II\(\alpha\) from the nucleus of cells maintained on a monolayer to the cytoplasm of cells grown in spheroid culture. In experiments examining the effects of adhesion on drug cytotoxicity in hematopoietic cancer cells, Hazlehurst et al. (16) demonstrated reduced mitoxantrone and etoposide-induced DNA double-strand breaks and apoptosis in adherent cells as compared with cells grown in suspension. This primarily \(\alpha\)\(\beta_i\)-mediated reduction in DNA damage correlated with decreased in topo II enzymatic activity. Further investigation showed that cellular adhesion correlated with a decreased salt extractability of topo II\(\beta_i\), suggesting that topo II\(\beta_i\) may be more tightly bound to DNA. Confocal microscopy revealed distinct punctate topo II\(\beta_i\) clusters in cells adhered to FN, compared with a diffuse nuclear distribution in cells maintained in suspension, demonstrating a link between the nuclear distribution of topo II\(\beta_i\) and cellular sensitivity to topo II inhibitors. Together, the studies by Oloumi et al. (17) and Hazlehurst et al. (16) indicate that alterations in subcellular localization of key chemotherapeutic targets may be an important regulatory mechanism by which cellular adhesion regulates sensitivity to cytotoxic agents.

**Decreased Apoptosis.** Adhesion has been demonstrated to directly regulate the apoptotic machinery. Although no direct evidence has linked the following regulatory mechanisms and MDR, the known role Bcl-2 family members in drug resistance suggests that they may also play a role in MDR (39, 41, 72–74). Gilmore et al. (75) demonstrated that the proapoptotic protein Bax is maintained in the cytosol in adherent mammary epithelial cells. Maintenance of cells in suspension resulted in a conformational change in Bax, facilitating the reversible translocation of Bax from the cytosol to the mitochondria and initiation of anokis (75). These data suggest that adhesion of mammary epithelial cells promotes cell survival by maintaining Bax in a nonapoptotic state in the cytosol. Integrin-associated signaling factors have also been demonstrated to regulate cell survival by directly targeting apoptotic machinery (38, 76–78). Datta et al. (76, 78) showed that phosphorylation of specific serines (Ser) of Bad facilitated the recruitment of 14-3-3 chaperone proteins to Bad. The association of 14-3-3 proteins with phospho-Bad facilitated a secondary Ser phosphorylation, decreasing Bad’s binding affinity for Bcl-2 or Bcl-X\(\text{L}\) and transport of the phospho-Bad/14-3-3 complex to the cytosol, promoting cell survival. These studies demonstrate that adhesion can modulate sensitivity to programmed cell death by direct regulation of apoptotic effectors. Interestingly, as with topo II\(\alpha\) and topo II\(\beta_i\), the regulation of Bax and Bad occurred via adhesion-mediated alterations in subcellular localization of apoptotic effectors, suggesting that redistribution of key apoptotic effectors may be an important mechanism by which cellular adhesion controls sensitivity to cytotoxic stress.

**Integrin-induced Signaling Cascades and Cytoskeletal Rearrangement.** The final class of adhesion-mediated MDR involves integrin-induced signaling cascades and cytoskeletal rearrangement. Meredith et al. (24) previously demonstrated that integrin-mediated phosphorylation events were required for the antiapoptotic effects of anchor-age-dependent growth. Corroborating these results, Sethi et al. (12) demonstrated that tyrosine phosphorylation was required for the protective effects of CAM-DR in small cell lung carcinoma cell lines. Sethi et al. (12) demonstrated that \(\beta_i\) integrin-mediated adhesion inhibited caspase-3 cleavage and apoptosis induced by a panel of chemotherapeutic drugs in small cell lung carcinoma cell lines. The authors showed that treatment of adherent cells with tyrophostine-25 (a protein tyrosine kinase inhibitor) blocked the protective effects of FN adhesion, indicating that integrin-mediated tyrosine phosphorylation was essential in regulating the antiapoptotic effects of cellular adhesion (12). No direct links have been identified between tyrosine phosphorylation and the categories of CAM-DR described above; however, this does not exclude the possibility that tyrosine phosphorylation is involved in their regulation. Another consequence of integrin ligation tightly associated with signaling is alterations in cytoarchitecture. There are several reports demonstrating that integrin signaling alone may not be sufficient to promote survival; cell attachment and geometry may also contribute to the prosurvival phenotype afforded by adhesion (79, 80).
Chen et al. (80) demonstrated in human endothelial capillary cells that not only were the signaling pathways initiated by interactions with ECM required for cellular homeostasis, but specific cellular geometry was also required for the protective affects mediated by adhesion. Chen et al. (80) demonstrated that serum deprivation-induced programmed cell death increased when cell spreading was attenuated by decreasing the spacing between ECM-coated micropatterned substrates. This report suggests that the survival-promoting effects of adhesion may require concomitant integrin-mediated signaling and integrin-mediated alterations in cytoarchitecture.

The studies summarized above demonstrate that direct contact between tumor cells and their environment confers a survival advantage to adherent cells. These interactions may provide initial or de novo mechanisms of resistance to tumor cells, facilitating further transforming mutations and the acquisition of MDR. Importantly, these results suggest that the environmental context of cancer cells may have important consequences on the acquisition of MDR and that therapies designed to disrupt specific interactions between cells and their environment may be important targets in the circumvention of MDR. Recently, using a combinatorial library designed against the laminin receptor (α6β1, integrin; Ref. 81), DeRoock et al. (82) identified several α-amino acid-containing synthetic peptides. The RZ-3 (kmvlywkg) peptide blocked adhesion of DU-H human prostate cancer cells to the ECM components FN, laminin-1, laminin-5, and collagen IV in a dose-dependent manner (82), indicating that it affected more that just α6β1 adhesion and possibly involved specific inhibition of the β1 integrin binding. These observations suggest that RZ-3 and similar peptides that interfere with cell-environment interactions may potentially reverse (or block) CAM-DR, thereby sensitizing cancer cells to chemotherapy.

Summary

Treatment of cancer with regimens of chemotherapy or radiotherapy invariably fails due to the development of MDR. In some cases, attempts to sensitize multidrug-resistant cancers result in initial success but inevitably fail due to the multifactorial nature of acquired MDR. The identification of CAM-DR, a phenomenon that elicits a de novo drug resistance, may play an important role as an early antisapoptotic effector allowing the emergence of acquired MDR. The characteristics of de novo CAM-DR may allow us to target therapies at a point prior to the emergence of acquired MDR. Several processes involved in cellular adhesion provide intriguing targets for the disruption of CAM-DR: (a) abrogation of direct cell contact (blocking peptides); (b) inhibition of small G protein prenylation (farnesyl transferase inhibitors and geranylgeranyl transferase inhibitors); and (c) interruption of farnesyl- and geranylgeranyl-PPi synthesis (amino-bisphophonates). In targeting these processes, we may be able to sensitize cancer cells to cytotoxic insult and, in turn, prevent the emergence of acquired mechanisms of MDR, facilitating a more successful treatment of cancer.
may be able to sensitize cancer cells to cytotoxic insult and, in turn, prevent the emergence of acquired mechanisms of MDR, facilitating a more successful treatment cancer. Lastly, in this review, we have discussed data demonstrating that cell adhesion confers a protective advantage to adherent cancer cells against classical chemotherapeutic agents and irradiation. Of note, we recently demonstrated that FN adhesion of the chronic myelogenous leukemia cell line K562 also reduced the effectiveness of the BCR-Abl kinase inhibitors STI-571 and AG957 (14). These data indicate that the cancer cell microenvironment provides a sanctuary conferring resistance to cancer therapy. Therefore, interactions between cancer cells and their environment may be critical targets in the success of many regimens of treatment.

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