Multidrug resistance: a role for cholesterol efflux pathways?

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Multidrug resistance (MDR) severely impairs the efficacy of cancer chemotherapy. Several protein transporters that mediate drug export have been identified, but additional adaptations appear to be necessary for full-fledged drug resistance. The cell surface density of caveolae and the expression of the caveolar coat protein caveolin are dramatically increased in MDR cancer cells. Acquisition of MDR might thus be accompanied by upregulation of caveolin-dependent cholesterol efflux pathways, raising the possibility that these same pathways are utilized for delivering drugs from intracellular compartments to the plasma membrane, where drugs can be extruded from the cells by drug efflux ATPases. The upregulation of caveolin mandates a phenotypic change of MDR cells in terms of their cholesterol homeostasis and is accompanied by loss of important features of the transformed phenotype of MDR cancer cells.

SYSTEMIC CHEMOTHERAPY WITH cytotoxic drugs is a widely used treatment that improves long-term survival of cancer patients. Yet, it has been estimated that only ~10% of chemotherapy-treated patients are ultimately cured1. The main reason for the limited efficacy of chemotherapy is multidrug resistance (MDR), defined as cellular resistance to multiple, structurally and functionally divergent drugs. MDR can be intrinsic (i.e. primary tumors are refractory to drug treatment) or acquired (i.e. tumor cells acquire drug resistance during treatment and are refractory on relapse). In many cases, MDR is believed to be due to overexpression of a drug transporter that mediates an energy-dependent efflux of drugs (or drug conjugates) across the plasma membrane, thus reducing intracellular drug concentration. P-glycoprotein (P-gp), product of the human MDR1 gene, is a prototypic drug efflux ATPase that has been studied extensively2. Numerous studies have demonstrated that P-gp mediates unidirectional drug transport, that it exhibits a drug-dependent ATPase activity, as well as ATP-dependent drug binding, and that its expression reduces cell sensitivity to drugs1,3. Nevertheless, it is clear that in many cases drug resistance conferred by transfected P-gp alone is significantly less efficient than that of drug-selected MDR cell lines (Ref. 4 and citations therein). Hence, although P-gp and other drug transporters are necessary for drug efflux across the plasma membrane, they are not always sufficient for a full-fledged MDR phenotype in cancer cells. Acquisition of MDR by selection with increasing drug concentrations appears, therefore, to involve additional mechanisms that contribute to cellular drug resistance. Indeed, it is now recognized that MDR cells and tumors employ diverse mechanisms to prevent or circumvent the lethal effects of chemotherapeutic drugs (Fig. 1).

Here we shall review recent results which suggest that cellular drug transport itself is more complex than previously thought and can involve the participation of caveolin-dependent cholesterol efflux pathways. It is postulated that these pathways can help deliver chemotherapeutic drugs from intracellular membranes to the plasma membrane against a steep concentration gradient. At the plasma membrane, drugs are extruded to the extracellular space by drug efflux pumps such as P-gp.

Cholesterol efflux pathways: role of caveolin and caveolae

Cellular cholesterol content is normally under strict homeostatic control, and mechanisms of cholesterol uptake, de novo synthesis and efflux are highly regulated5. Cholesterol efflux pathways have been a focus of much recent attention, as studies on protein and cholesterol transport converged, pointing at cholesterol-rich membrane microdomains or proteolipid complexes, or both, as carriers of newly synthesized free cholesterol to the plasma membrane (Fig. 2)6,8. Cellular cholesterol is accrued by: (i) internalization of intact low-density lipoprotein (LDL) carrying cholesteryl ester by endocytosis via high-affinity LDL receptors; (ii) selective uptake of free cholesterol by monolayer exchange, mainly from LDL; (iii) selective uptake of cholesteryl ester by exchange, mainly from HDL; and (iv) de novo synthesis of cholesterol by the mevalonate pathway in the endoplasmic reticulum (ER)6,7. Several lines of evidence suggest that the pathways involved in transport of protein and cholesterol from the ER to the plasma membrane are different, although many uncertainties remain, especially with regard to the nature of the cholesterol carriers (or transport intermediates)8.

The transport of nascent cholesterol to the plasma membrane was studied by pulse-chase metabolic labeling, followed by isolation of plasma membranes or desorption of plasma membrane cholesterol by acceptors such as lipoproteins or cyclodextrins. By most accounts, transport of labeled cholesterol to the plasma membrane is rapid ($t_{1/2}$ ~10–30 min.) and energy dependent6–14. As opposed to the vesicular transport of proteins, such as vesicular stomatitis virus G protein and influenza virus hemagglutinin, cholesterol transport is affected neither by agents that disrupt the cytoskeleton (e.g. cytochalasin B) nor by agents that disrupt the Golgi apparatus (e.g. brefeldin A)10–14. These data support a model in which the transport of newly synthesized cholesterol to the plasma membrane is mostly nonvesicular and does not require an intact Golgi apparatus. However, as cholesterol is elevated in Golgi membranes relative to the ER and there is a gradient of increasing cholesterol concentration from the cis-Golgi to the trans-Golgi15, it would appear that transport of newly synthesized cholesterol via the Golgi does occur under physiological conditions, even if cholesterol can bypass the Golgi when the latter is pharmacologically disrupted. The Golgi is supplied also by cholesterol acquired from plasma LDL and routed to the Golgi via lysosomes8.

How is cholesterol carried from the ER and Golgi to the plasma membrane? What is the nature of its transport
intermediates? A small fraction of cholesterol is likely to take the vesicular route and be carried by clathrin- and COP-coated exocytic vesicles, if only because it is difficult to envision how it could be totally excluded from such vesicles. In addition to clathrin- and COP-coated vesicles, the trans-Golgi network (TGN) sorts certain classes of membrane proteins (e.g. fatty acylated and glycosylphosphatidylinositol (GPI)-anchored proteins) into cholesterol- and sphingolipid-rich membrane microdomains that have been termed rafts (Ref. 16; see Box 1 for a brief discussion of cholesterol-rich microdomains, rafts and caveolae). Raft-associated proteins are targeted to the plasma membrane in nonpolarized cells and, preferentially, to the apical plasma membrane in polarized cells16. Because of their high cholesterol content, rafts are likely to play an important role in delivering cholesterol to the plasma membrane. Supporting this hypothesis are data showing that traffic of both LDL-derived and de novo synthesized cholesterol to the plasma membrane is inhibited by nocodazole and numerous other amphiphiles that block raft-dependent protein transport6,11.

Recent work shows that newly synthesized cholesterol associates with intracellular detergent-insoluble lipid rafts before it appears on the cell surface14. Together with data on the emergence of detergent-insolubility in GPI-anchored proteins and glycosphingolipids17, these results suggest that assembly of lipid rafts occurs intracellularly. The lack of detailed spatial and temporal information on raft assembly and maturation notwithstanding, and although the exact physical nature of the rafts assembled in the ER and Golgi is unknown, lipid rafts emerge as quantitatively major carriers of cholesterol to the plasma membrane. At the plasma membrane, cholesterol newly arrived from the TGN is initially concentrated in cholesterol-rich membrane domains that are enriched with caveolin, and then diffuses to other, cholesterol-poor domains of the plasma membrane10,12.

The major caveolar coat protein caveolin-1 is strongly implicated in cholesterol efflux. Caveolin-1 binds cholesterol18 and requires cholesterol for efficient reconstitution into liposomes18,19 and for oligomerization with other caveolin molecules20. Transfection of caveolin-1 into lymphocytic cells (that normally lack caveolin) results in a fourfold increase in the rate of transport of newly synthesized cholesterol to caveolar membranes and a fivefold elevation of cholesterol mass in these membranes12. Transfection of caveolin-1 upregulates cholesterol efflux also in human skin fibroblasts21. Conversely, expression of a dominant-negative caveolin mutant appears to compromise the integrity of caveolae by interfering with the transport of cholesterol22. In addition, the level of endogenous caveolin-1 and the rate of free cholesterol efflux are directly correlated under certain physiological conditions (e.g. the cell cycle in fibroblasts21 and during macrophage differentiation23). Such a correlation exists also following certain pharmacological manipulations10,12,24. Hence, caveolin is likely to play a direct role in cholesterol transport to the plasma membrane. This conclusion is supported, albeit indirectly, by the fact that caveolin-1 expression is transcriptionally regulated by the sterol-regulatory-element-binding protein (SREBP). Consistent with the presumed role of caveolin in mediating cholesterol efflux, SREBP downregulates its expression25. This effect is opposite in direction to the well-known stimulatory effect of SREBP on sterol biosynthetic enzymes and LDL receptor expression.

How does caveolin mediate cholesterol efflux? One possibility is that caveolin oligomers, which form soon after its synthesis, are assembled into caveolin-containing, cholesterol-rich lipid rafts at the TGN, where an intracellular pool of caveolin has been identified26. These caveolin-rich rafts (termed “precaveolae”)27 must then be transported to the plasma membrane where they combine to form invaginated caveolae. Alternatively, a caveolin-1-chaperone complex comprising heat-shock protein 56, cyclophilin 40 and cyclophilin A, that carries cholesterol to the plasma membrane was recently demonstrated11.

Pulse-chase and subcellular fractionation experiments indicate that caveolae act as plasma membrane terminals from where free cholesterol flows to noncaveolar regions of the membrane or is unloaded onto HDL10,12. The scavenger receptor class B type I (SR-BI) is localized in caveolae where it mediates cholesterol-rich lipid raft uptake, if only because it is directly correlated under certain physiological conditions (e.g. the cell cycle in fibroblasts21 and during macrophage differentiation23). In summary, the above data suggest that a cholesterol efflux pathway exists, which is upregulated in caveolin-1-expressing cells. Caveolin-1 might play multiple roles. First, as a component of TGN-derived lipid rafts (whatever physical form those may assume) and of cytosolic caveolin–chaperone complexes, which are both targeted to the plasma membrane. Second, as a structural coat protein of plasma membrane caveolae, which constitute a site where desorption of cellular cholesterol by HDL takes place and thus represent the last workstation in its journey out of the cell.
Cellular cholesterol trafficking: a hypothetical role of cholesterol efflux pathways in drug export. Major cholesterol influx pathways (blue arrows) and efflux pathways (green arrows) are depicted schematically. Cellular cholesterol is derived from several sources7,8. De novo synthesis of cholesterol occurs in the endoplasmic reticulum (ER; thick green arrow). Cholesterol ester (dashed arrows) is acquired from low-density lipoproteins (LDL) by internalization via clathrin-coated pits and, following hydrolysis in lysosomes, is routed to the plasma membrane and to the ER, a transport step that depends on the Niemann–Pick C1 protein. HDL-derived cholesteryl ester is acquired by selective uptake, a process mediated by the scavenger receptor-BI (SR-BI) that resides in cholesterol-rich domains of the plasma membrane and in invaginations, termed caveolae (orange dotted)23,28,29. Free cholesterol (solid arrows) is taken up by the plasma membrane via monomer exchange, mainly from LDL. Plasma membrane cholesterol is recycled back to the Golgi. Regardless of the source, cholesterol accumulates in the trans-Golgi network (TGN) on en route to the plasma membrane. TGN-to-plasma-membrane transport of cholesterol can occur in small part via a vesicular pathway. However, recent evidence implicates two caveolin-1-dependent routes as major pathways for TGN to plasma membrane transport of cholesterol, one involving caveolin-containing lipid rafts and another involving caveolin-1 complexes with chaperones (see text for details). Caveolae are likely to serve as terminals for rafts and caveolin–chaperone complexes. An ATP-binding cassette transporter (ABC1) has been identified recently that can act as a flipase to facilitate the transbilayer movement of cholesterol across the plasma membrane, although its localization in caveolae has not yet been demonstrated47. Cholesterol is unloaded onto HDL via its SR-BI receptors that reside in caveolae and rafts. Lipophilic drugs enter cells by passive diffusion (solid red arrows) and partition into various intracellular cell membranes. To be extruded by plasma membrane transporters the drugs must first be transported from intracellular compartments to the plasma membrane against a steep concentration gradient (dashed red arrows). It is hypothesized that drugs are delivered to the plasma membrane on the same platforms (i.e. caveolin-containing rafts and caveolin–chaperone complexes) that transport cholesterol. Drugs can then be extruded by drug transporters, such as P-glycoprotein, that are localized in plasma membrane cholesterol-rich domains37,43.

Caveolin in MDR cancer cells: MDR cells as cholesterol homeostasis type B cells

Mammalian cells have been classified6 in one of two types on the basis of the manner in which their cholesterol homeostasis is regulated (Table 1). In type A cells, cholesterol homeostasis is regulated mainly at the level of influx. Type A cells were defined as cells that have few cell surface lipoprotein receptors but many caveolae6. Type B cells include adipocytes, pneumocytes, quiescent fibroblasts, endothelial cells, epithelial cells and smooth muscle cells. In terms of their cholesterol metabolism and transport, most oncogenically transformed and cancer cells have been classified as type A cells (i.e. cells that have few or no caveolae)6. Downregulation of caveolin expression occurs upon oncogenic transformation of the cells30, and, with few exceptions (Ref. 31), levels of caveolin-1 in most human cancer cells are very low and often undetectable12–34. The loss of caveolin expression in cancer cells has important functional consequences, because when caveolin-1 is reintroduced into transformed cells by transfection, the cells are no longer capable of anchorage-independent growth and become nontumorigenic25,35. Furthermore, antisense inhibition of caveolin-1 expression in normal fibroblasts is sufficient to induce cell transformation, allowing anchorage-independent growth in vitro and tumor formation in vivo36.
In view of the profound influence that caveolin has on the transformed phenotype, recent results showing that MDR cancer cells express very high caveolin levels and exhibit a high surface density of caveolae were quite unexpected. Expression of caveolin-1 is dramatically upregulated in numerous MDR human cancer cell lines, including adriamycin-resistant MCF-7 AdrR breast adenocarcinoma cells and colchicine-resistant HT-29-MDR colon carcinoma cells; vinblastine-resistant SKVLB1 ovarian carcinoma cells and taxol-resistant A549-T24 lung carcinoma cells; and colchicine-resistant B16-MDR mouse melanoma cells. Thus, various cell types, while developing resistance to different drugs, acquire high caveolin expression as well as high surface density of caveolae. This is consistent with previous studies showing that MDR is associated with elevated levels of cellular glycosylceramide. Glucosylceramide was recently shown to be a major glycosphingolipid constituent of caveolar membranes. Importantly, the parallel up-regulation of caveolin and P-gp in MDR cells is not interdependent, because transfection with neither protein leads to changes in the expression of the other (G. Fiucci and M. Liscovitch, unpublished).

One immediate implication of the up-regulation of caveolin and caveolae in MDR cells is that during acquisition of the MDR phenotype the cells revert from being cholesterol homeostasis type A cells (containing little or no caveolin, typical of transformed cells) to cholesterol homeostasis type B cells (with a high caveolin level, typical of differentiated cells). This phenotypic change has yet to be examined directly. However, in addition to the caveolin data, it is supported by the fact that LDL receptor and MDR1 expression in CEM and MOLT4 cells are inversely correlated. It is also consistent with other data showing that MDR cells have lower LDL uptake compared with the parental drug sensitive cells, suggesting lower LDL receptor expression. It might thus be reasonable to assume that acquisition of the MDR phenotype is accompanied by acquisition of a type B cholesterol homeostasis phenotype. Being type B cells, MDR cancer cells must have an active cholesterol efflux pathway. Why should this phenotypic change confer any selective advantage to the cells, and how could it contribute to their survival in the presence of cytotoxic drugs?

Potential role of cholesterol efflux pathway(s) in delivering drugs to plasma membrane drug efflux pumps

For a cytotoxic drug to be extruded by plasma membrane resident P-gp (or other drug transporters), it must first be efficiently transported to the plasma membrane from the various intracellular membranes into which it partitions. It could be postulated that an intracellular cholesterol efflux pathway can deliver cytotoxic drugs (most of which are lipophilic compounds) to the plasma membrane upon the same platforms ('transport intermediates') that deliver cholesterol and sphingolipids (Fig. 2). A highly active cholesterol efflux pathway could increase the efficiency of overall drug transport in two ways. First, by accelerating the transport of drugs from intracellular membranes to the plasma membrane against a steep concentration gradient. Second, by delivering drug-bearing rafts and caveolin–chaperone complexes directly to plasma membrane cholesterol-rich domains. The latter is important, because this is where P-gp is localized, thus resulting in increased transport of plasma membrane cholesterol to the endoplasmic reticulum, where it is esterified.

Certain amphiphilic compounds that inhibit the ATPase activity of P-gp and ATP-dependent drug export are known also to perturb various aspects of cholesterol transport and metabolism. These amphiphiles (progesterone, verapamil, tri-fluperazine, etc.) inhibit esterification of plasma membrane-derived cholesterol and block a late step in cholesterol biosynthesis, resulting in the accumulation of lanosterol and other cholesterol precursors. It has therefore been suggested that P-gp is involved in regulation of cholesterol homeostasis. However, amphiphiles used in these studies are not specific inhibitors of P-gp. The correlation between expression of P-gp and perturbation of cholesterol esterification and synthesis, by more specific inhibitors of P-gp, were addressed in a recent study that utilized parental and MDR myeloma cells and P-gp-transfected fibroblasts. This work has demonstrated that the effect of specific P-gp inhibitors on cholesterol esterification depends on cell type and is, by and large, independent of P-gp expression, thus negating a direct role for P-gp in regulation of cholesterol homeostasis. Overexpression of P-gp, whether in P-gp-transfected fibroblasts or in MDR myeloma cells, does however lead to a small increase in cholesterol esterification. This effect can be related to the recycling of plasma membrane P-gp through a constitutive, Rab5-dependent endocytic pathway. This process is likely to be associated with elevated turnover of cholesterol-rich membranes (in which P-gp is localized), thus resulting in increased transport of plasma membrane cholesterol to the endoplasmic reticulum, where it is esterified.

Reversal of the oncogenic phenotype in MDR cancer cells

What might be the impact of the phenotypic change of type A cancer cells into type B MDR cells on cancer cell biology? By expressing caveolin-1, MDR cancer cells reacquire a prominent marker of nontransformed cells that has been lost upon oncogenic transformation. Given the dramatic effects of caveolin-1 expression on the phenotype of transformed cells, this fact is
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likely to have important consequences. Indeed, we have recently found that MCF-7 AdrR cells have lost the capacity for anchorage-independent growth, indicating that they have regained an essential requirement for an extracellular matrix-derived survival or growth signal, or both (G. Fiucci, R. Reich and M. Liscovitch, unpublished). Previously, it has been shown that certain MDR cell lines exhibit reduced tumorigenicity in athymic mice. It thus seems that the acquisition of MDR, while allowing cells to survive the high concentrations of cytotoxic agents during chemotherapy, is often accompanied by loss of at least some features of the transformed phenotype. This change may prove to be a previously unsuspected Achilles heel of MDR cells that will enable future development of much needed novel drugs for treatment of MDR cancer.

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