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Advanced Drug Delivery Reviews 49 (2001) 317–323

Advanced DRUG DELIVERY Reviews

www.elsevier.com/locate/drugdeliv

Upregulation of caveolin in multidrug resistant cancer cells: functional implications

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Received 23 September 2000; accepted 3 April 2001

Abstract

Multidrug resistance (MDR) is a multifactorial process that involves elevated expression of drug transporters as well as additional biochemical changes that contribute to the drug resistant phenotype. Here we review recent results indicating the upregulation of constituents of rafts and caveolae, including glucosylceramide, cholesterol and caveolin-1, in MDR cells. Accordingly, the number of plasma membrane caveolae is greatly increased in MDR cells. The relationship between caveolin and MDR may be linked to the function of caveolin-1 in mediating cholesterol efflux, a pathway that we hypothesized to facilitate the delivery of drugs from intracellular compartments to plasma membrane resident drug transporters. An additional link seems to exist between the upregulation of GlcCer synthase and attenuation of ceramide-mediated apoptotic signaling. These adaptations may promote cell survival during chemotherapy and, hence, would be positively selected during cell exposure to cytotoxic drugs. However, the overexpression of caveolin-1, an oncosuppressive protein, may also reverse or attenuate important aspects of the phenotypic transformation of MDR cells. The molecular mechanisms by which caveolin-1 exerts its effects on cell proliferation, cell survival, and multidrug resistance remain to be fully elucidated.

Keywords: Cancer; Caveolae; Caveolin; Cholesterol; Glucosylceramide; Multidrug resistance; Rafts; Transformation

Contents

Abbreviations: GlcCer, glucosylceramide; MDR, multidrug resistance; P-gp, P-glycoprotein; SREBP, sterol regulatory element-binding protein

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0169-409X/01/$ – see front matter
PII: S0169-409X(01)00144-2

1. Introduction

Although first noted as a highly tyrosine-phosphorylated 21-kDa protein in v-Src-transformed cells [1,2], caveolin has received much of the attention it has attracted (and its name) because it serves as a major coat protein of caveolae [3,4]. The caveolar membrane system, comprising plasma membrane invaginations and juxtamembrane vesicle clusters, is thought to be involved in a number of protein transport processes, including transcytosis, potocytosis, and other clathrin-independent endocytic processes [5]. In addition, recent work implicates caveolin and caveolae in pathways of cholesterol efflux from the cells [4,6].

2. Changes in caveolin expression during cell differentiation and oncogenesis

Caveolae are found in particularly large numbers at or near the plasma membrane of differentiated epithelial cells (e.g. pneumocytes and endothelial cells), fibroblasts, smooth muscle cells and adipocytes. Adipose tissue is particularly enriched in caveolae, and caveolin mRNA and protein are strongly induced upon differentiation of 3T3-L1 fibroblasts to adipocytes [7]. Previous reports of little or no caveolin expression in neuronal cells notwithstanding, recent work has shown an upregulation of caveolin-1 and -2 expression in differentiating PC-12 cells and dorsal root ganglion neurons [8]. Caveolin-1 expression is also upregulated when progenitor lung cells acquire an alveolar epithelial type I phenotype in vitro [9]. Likewise, the expression of caveolin-1 is high in differentiated Schwann cells, whereas it is decreased when the cells revert to a premyelinating phenotype [10]. In summary, the differentiation of various cells is frequently associated with elevated expression of caveolin proteins and caveolae organelles.

In contrast to the upregulation of caveolin-1 expression during cell differentiation, the protein appears to be down-regulated in oncogenically-transformed cells and its expression is low in most (but not all) cancer cells. Caveolin-1 was identified as one of several gene products that are strongly down-regulated in human mammary carcinomas [11]. The caveolin-1 gene is localized to a suspected tumor suppressor locus in human chromosome 7 (7q31.1/D7S522) that is deleted in some forms of cancer [12,13]. Caveolin-1 mRNA and protein expressions are reduced or absent in most oncogenically transformed cell lines and tumor cell lines examined [14–18]. Furthermore, at least in certain cell types, antisense inhibition of caveolin-1 expression is sufficient for induction of oncogenic transformation, as shown in NIH 3T3 fibroblasts [19].

The above data are consistent with a simple model according to which caveolin-1 acts as a tumor suppressor protein. However, another analysis has shown that while most breast, pancreatic and ovarian carcinoma cells do express little or no caveolin-1, bladder carcinoma cells exhibit high caveolin-1 expression [18]. Also, over 78–93% of malignant human breast tissues, including intraductal carcinoma, infiltrating ductal carcinoma and lymph node metastasis, were positively immunostained with a caveolin-1 antibody [20]. These data stand in contrast to results obtained in breast cancer cell lines [15,16,18]. In prostate cancer tissue, the percentage of caveolin-1-positive samples was only 29% in T_3N_1-stage primary cancer and increased to 56% in lymph node metastasis [20]. Hence, down-regulation of caveolin-1 may be sufficient but is not an essential condition for cellular transformation.

The proposed oncosuppressive activity of caveolin-1 is supported by data showing that expression of caveolin-1 inhibits mitogenic signaling [16,21]. Engelman et al. have shown that recombinant expression of caveolin-1 in oncogenically transformed NIH-3T3 cells abrogates anchorage-independent growth, depriving the transformed cells of one of their crucial tumorigenic abilities [22]. Likewise, transfection of human mammary cancer cells with caveolin-1 cDNA results in substantial growth inhibition as demonstrated by a 50% decrease in growth rate and a $\sim$15-fold reduction in colony formation in soft agar [15]. Additional information from this study indicated that caveolin-1 expression is regulated during the cell cycle [15]. Collectively, the above data indicate that caveolin-1 has growth-inhibitory activities that may be repressed during oncogenic transformation and tumorigenesis. As noted above, such repression is not obligatory and

caveolin-1-positive cancer cells exist where these inhibitory activities are presumably bypassed.

The mechanisms by which caveolin-1 inhibits cell proliferation involve its multifarious interactions with growth-promoting signaling molecules. Many of the molecules that comprise mitogenic signaling pathways were localized to caveolae or were shown to physically interact with caveolin-1 [4]. It has been suggested that caveolin forms a scaffold onto which the signaling molecules can assemble and thus co-localize within a distinct domain of the plasma membrane (i.e. caveolae), from which signaling cascades might be launched [23]. The binding of caveolin-1 to these molecules (via the so-called caveolin scaffolding domain) may regulate the function of these molecules. Caveolin-1 exerts an inhibitory effect on the activity of H-Ras, c-Src, Raf-1, Mek, Erk2, EGF-R (ErbB1) and ErbB2, among others [4,23]. It may thus be concluded that caveolin-1 exhibits a general pattern of negative modulation on oncogenic pathways leading to cell proliferation.

3. Mechanisms of multidrug resistance in cancer

Multidrug resistance (MDR) is defined as cellular resistance to multiple, structurally and functionally divergent drugs [24]. The efficacy of cancer chemotherapy is severely limited by MDR. Cells often employ more than one mechanism in order to survive the cytotoxic insult of drugs utilized in chemotherapy. P-glycoprotein (P-gp) and related ATP-dependent drug efflux pumps mediate drug resistance by actively extruding drugs from the cells [24–28]. MDR may also be conferred by altered pharmacokinetics of drug uptake into cells or by an increased rate of drugs metabolism (detoxification). In addition, the cell response to the drug may be altered, e.g. by stimulated repair of DNA damage and/or suppression of drug-induced apoptosis [29,30]. Two or more cellular mechanisms often act concurrently to confer the characteristic cross-resistance to various drugs displayed by MDR cells [29,30].

Among the many cellular changes that accompany the multidrug resistant phenotype are prominent changes in cell lipid composition and metabolism. Recent studies have shown that acquisition of the MDR phenotype is associated with upregulation of lipids that constitute lipid rafts and caveolar membranes, notably glucosylceramide and cholesterol. These studies were reviewed recently elsewhere [31]. Of particular interest are the elevated cellular levels reported for cholesterol (in some but not all MDR cell lines) and for GlcCer [32–34]. Both cholesterol and GlcCer are enriched in the membrane microdomains termed lipid rafts and in caveolae [4,35,36]. The elevated levels of GlcCer and cholesterol, independently reported in different types of MDR cells, suggest that MDR might be associated with increased formation of these microdomains in the plasma membrane. Indeed, recent work has provided direct evidence showing that caveolae and the caveolar coat protein caveolin are upregulated in MDR cells.

4. Upregulation of caveolin in MDR cells

Multidrug resistant cancer cells express very high caveolin levels and exhibit a high surface density of caveolae. This has been shown recently in numerous MDR human cancer cell lines [37,38]. The high expression of caveolin-1 was demonstrated in adriamycin-resistant MCF-7 AdrR breast adenocarcinoma cells and colchicine-resistant HT-29-MDR colon carcinoma cells [37], and in vinblastine-resistant SKVLB1 ovarian carcinoma cells and taxol-resistant A549-T24 lung carcinoma cells [38]. In addition, we have made similar observations in colchicine-resistant B16-MDR mouse melanoma cells (J. Troost, G. Faiman, unpublished results). The expression of caveolin-2 is upregulated too, as observed in HT-29-MDR, MCF-7 AdrR, and B16-MDR cells [37] (J. Troost, G. Faiman, unpublished results). Thus, the acquisition of resistance to different drugs by various cell types is associated with high expression of caveolin. In HT-29-MDR cells the upregulation of caveolin-1 is accompanied by a five-fold increase in the density of juxtamembrane, 50 to 100-nm non-coated invaginations that exhibit the characteristic flask-shaped morphology of caveolae. A comparable surface density of caveolae was also observed in MCF-7 AdrR cells, whereas the parental MCF-7 cells were practically devoid of caveolae [37].

5. Possible implications of high GlcCer level and caveolin expression in MDR cells

The changes in lipid and protein constituents of caveolae could contribute to the drug resistance phenotype in more than one way. The elevated levels of GlcCer in MDR cells appear to be due to higher activity of GlcCer synthase, the enzyme which glucosylates ceramide to form GlcCer [32,33]. Recent follow-up work has demonstrated that overexpression of recombinant GlcCer synthase confers resistance to adriamycin and to ceramide in human breast cancer cells [39]. Drug-induced apoptosis involves an increase in cellular ceramide levels [40]. It was therefore suggested that drug resistance in these GlcCer synthase-transfected cells might be related to stimulation of glucosylation of ceramide and the consequent attenuation of the apoptotic signal promoted by the chemotherapeutic drug [39]. In addition, accumulation of GlcCer may enhance cell survival upon exposure to chemotherapy. The contribution of GlcCer synthase to drug resistance was demonstrated directly by antisense inhibition of GlcCer synthase expression, which resulted in a 30% decrease in GlcCer synthase activity and a 28-fold increase in adriamycin sensitivity [41]. These results strongly support the hypothesis that GlcCer synthase contributes to drug resistance in MDR cells by clearing the ceramide that carries a drug-induced apoptotic message.

The role of caveolae in drug resistance is still unclear. Several cellular functions were assigned to caveolae, yet their proposed role in mediating cholesterol efflux seems most relevant in the context of MDR. The evidence in favor of a role for caveolin-1 in the transport of cholesterol to the plasma membrane was reviewed recently [4,6] (also refer to the article by Fielding and Fielding, this series). Briefly, the rate of transport of newly synthesized cholesterol to caveolar membranes is increased following transfection of caveolin-1 resulting in a significant increase in cholesterol mass in these membranes [42,43]. Consistently, a dominant negative caveolin mutant appears to disrupt caveolar membranes by interfering with the transport of cholesterol [44]. There is a direct correlation between caveolin-1 expression and free cholesterol efflux rate under various physiological conditions [43,45] and following certain pharmacological manipulations [42,46,47]. Caveolin-1 expression is transcriptionally regulated by the sterol regulatory element-binding protein (SREBP). SREBP downregulates caveolin-1 expression [48], an effect that is opposite in direction to the well known stimulatory effect of SREBP on sterol biosynthetic enzymes and low-density lipoprotein receptor expression. Caveolin-1 is a cholesterol-binding protein [49–51]. A soluble caveolin-1–chaperone complex that contains cholesterol, heat shock protein 56, cyclophilin 40 and cyclophilin A was recently demonstrated [52] (also refer to article by Matveev et al. in this series). 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 high-density lipoprotein molecules that interact with the scavenger receptor class B, type I [42,45,46,53,54]. In summary, these data suggest that a cholesterol efflux pathway exists that is up-regulated in caveolin-1-expressing cells. In this pathway caveolin-1 appears to act both as a component of plasma membrane-targeted cholesterol transport intermediates and as a structural coat protein of plasma membrane caveolae.

We have recently proposed that caveolin-dependent cholesterol efflux pathway(s) potentially may play a role in delivering drugs, from the various intracellular membranes into which they partition, to plasma membrane drug efflux pumps [55]. We hypothesized that an intracellular cholesterol efflux pathway could carry cytotoxic drugs (most of which are lipophilic compounds) upon the same platforms that deliver cholesterol and sphingolipids. If that were the case, a highly active cholesterol efflux pathway would facilitate drug transport by accelerating the transport of drugs from intracellular membranes to the plasma membrane against a steep concentration gradient and by delivering drug-bearing rafts and caveolin–chaperone complexes directly to plasma membrane cholesterol-rich domains. The latter is important because P-gp is localized in cholesterol- and caveolin-rich domains [37,56] (G. Fiucci et al., unpublished results). The hypothesis that efficient drug transport is a two step process, requiring both an active intracellular cholesterol efflux pathway *and* a plasma membrane drug efflux

pump, provides a rational explanation for the up-regulation of caveolin in MDR cancer cells.

Interestingly, as noted above caveolin-1 expression is higher in metastasis-derived prostate cell lines relative to primary tumor-derived prostate cell lines [20]. Subsequent analysis of mouse and human prostate specimens revealed markedly increased accumulation of caveolin-1 in lymph node metastasis [20]. These results correlate caveolin gene expression with other genes that are associated with prostate cancer progression. Thus, caveolin-1 expression may be linked to an increased clinical virulence of prostate cancer disease. An analogy may be drawn between the presumed caveolin-1-induced survival capacities in metastatic prostate cancer cells and in MDR cancer cells. One possibility which requires further study is that metastatic prostate cancer cells that express caveolin-1 are intrinsically more resistant to chemotherapeutic drugs. Such resistance may be related to the recently reported caveolin-dependent suppression of c-myc-induced apoptosis in prostate cancer cells [57].

6. Impact of caveolin-1 on the transformed phenotype of MDR cells

While the elevated expression of caveolin-1 may contribute to the MDR phenotype, caveolin may have parallel, dramatic 'side' effects on the transformed phenotype of the MDR cells. In effect, by expressing caveolin-1, multidrug resistant cancer cells reacquire a characteristic marker of non-transformed cells that is usually lost upon oncogenic transformation. Given the oncosuppressive actions of caveolin-1 [15,19,22], this fact is likely to have important consequences. Recent work suggests that caveolin-1 expression levels are inversely correlated with cyclin-D1 levels in transformed cells [58]. Cyclin-D1 protein is linked to positive regulation of cellular transformation. Thus, it might be that reversing the transformed cell phenotype in MDR cells by caveolin involves transcriptional repression of cyclin-D1 gene, which normally contributes to inhibition of proliferation and transformation. signals. In addition, we have recently found that multidrug resistant 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 and/or growth signal (G. Fiucci, M. Liscovitch, unpublished data). Previously it has been shown that certain MDR cell lines exhibit reduced tumorigenicity in athymic mice [59,60]. It thus seems that the acquisition of multidrug resistance, while allowing cells to survive in the presence of high concentrations of cytotoxic agents, 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 multidrug resistant cells that may enable future development of much needed novel drugs for treatment of multidrug resistant cancer.

Acknowledgements

We thank Drs. Malgorzata Czarny and Gabriel Faiman for many helpful discussions. We are grateful to Yona Eli and Tovi Harel-Orbital for excellent technical assistance. Our work is supported in part by grants from the United States–Israel Binational Science Foundation and the Israel Science Foundation. M.L. is the incumbent of the Harold L. Korda Professorial Chair in Biology.

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