NIH Public Access
Author Manuscript
Cell Rep. Author manuscript; available in PMC 2012 October 02.

Published in final edited form as:
Cell Rep. 2012 September 27; 2(3): 580–590. doi:10.1016/j.celrep.2012.08.011.

Anti-cancer activity of the cholesterol exporter ABCA1 gene

Bradley Smith and **Hartmut Land***
Department of Biomedical Genetics, University of Rochester Medical Center, 601Elmwood Ave., Rochester NY 14642, USA

Summary

The ABCA1 protein mediates the transfer of cellular cholesterol across the plasma membrane to apolipoprotein A-I. Loss-of-function mutations in the ABCA1 gene induce Tangier disease and familial hypoalphalipoproteinemia, both cardio-vascular conditions characterized by abnormally low levels of serum cholesterol, increased cholesterol in macrophages and subsequent formation of vascular plaque. Increased intra-cellular cholesterol levels are also frequently found in cancer cells. Here we demonstrate anti-cancer activity of ABCA1 efflux function, which is compromised following inhibition of ABCA1 gene expression by oncogenic mutations or cancer-specific ABCA1 loss-of-function mutations. In concert with elevated cholesterol synthesis found in cancer cells, ABCA1 deficiency allows for increased mitochondrial cholesterol, inhibits release of mitochondrial cell death-promoting molecules and thus facilitates cancer cell survival, overall suggesting that elevated mitochondrial cholesterol is essential to the cancer phenotype.

Introduction

Current interest in cancer cell metabolism originates from several recent discoveries indicating that elevated glycolytic activity found in various types of cancer, commonly known as the Warburg effect (Warburg, 1930), is an essential feature of the cancer phenotype (Christofk et al., 2008; Fantin et al., 2006; Weinberg et al., 2010). Similarly, a causal role for increased cholesterol in malignant cell transformation has long been debated, first suggested perhaps by work in the early 20th century indicating accelerated tumor growth following cholesterol injection into xenografts (Robertson and Burnett, 1913). Genetic evidence for a causal link between multi-step oncogenesis and the control of cholesterol homeostasis, however, has ever been lacking.

Aberrant regulation of cholesterol homeostasis has been associated with multiple types of cancer. Numerous studies have shown increased levels of cholesterol in tumors as compared to normal tissue (Dessi et al., 1992; Dessi et al., 1994; Kolanjiappan et al., 2003; Rudling and Collins, 1996; Schaffner, 1981; Yoshioka et al., 2000). Moreover, low serum cholesterol levels have been associated with tumor presence in cancer patients, suggesting cholesterol may accumulate in tumor tissue (Benn et al., 2011; Jacobs et al., 1992; Strasak et al., 2009). Multiple paths to increasing intracellular cholesterol have been observed in cancer cells. These include up-regulation of HMG-CoA reductase activity, the rate-limiting step of the cholesterol synthesis pathway (Caruso et al., 2002; Caruso et al., 1999; Notarnicola et al.,

© 2012 Elsevier Inc. All rights reserved.

*Correspondence: Tel: 585-273-1442, FAX: 585-273-1450, land@urmc.rochester.edu.

The authors have no conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

2004), loss of feed-back inhibition of HMG-CoA reductase by cholesterol (Gregg et al., 1986; Hentosh et al., 2001; Siperstein, 1995), increased uptake of extracellular cholesterol through the LDL receptor (Graziani et al., 2002; Schimanski et al., 2009; Tatidis et al., 2002), and decreased expression of the cholesterol exporter termed ATP binding cassette transporter A1 (ABCA1) (Basso et al., 2005; Ki et al., 2007; Moustafa et al., 2004; Schimanski et al., 2009), all together suggesting that cholesterol levels in cancer cells can be modulated by any of these interconnected processes.

In vivo evidence to support a cancer-promoting role of cholesterol is limited to pharmacological approaches. Various statins, which inhibit cholesterol synthesis by blocking activity of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoAR), have been shown to reduce tumor growth in xenograft models (Gao et al., 2010; Huang et al., 2010; Kochuparambil et al., 2011). Moreover, pharmacological inhibition of squalene synthase, the first committed step in cholesterol synthesis, lowered resistance to the chemotherapeutic agent doxorubicin in a xenograft model of liver cancer, albeit without significant effect on tumor growth, when used on its own (Montero et al., 2008). Furthermore, liver x receptor (LXR) agonists, which are known to induce ABCA1 expression, inhibited tumor growth and progression to androgen independence in a xenograft model of prostate cancer (Chuu et al., 2006). The anti-tumor effects of these compounds, however, may involve a variety of mechanisms, as for example, statins inhibit GTPases such as Ras and Rho family proteins via blocking protein prenylation and/or farnesylation (Demierre et al., 2005) and LXR agonists induce cell cycle arrest through up-regulation of p27 (Chuu and Lin, 2010).

Efforts to identify causal relationships between oncogenic mutations and associated metabolic or cholesterol dysregulation have been limited, presumably for two reasons: (1) most cancer mutations have been identified in genes regulating cellular signaling pathways (Pickeral et al., 2000), and (2) analysis of regulatory processes downstream of oncogenic mutations has been difficult due to the complexity of change associated with malignant transformation. Only recently have several genetic approaches been developed readily capable of identifying non-mutant genes essential to cell transformation (non-mutant drivers) (Luo et al., 2009; McMurray et al., 2008; Schlabach et al., 2008; Shaffer et al., 2008; Zender et al., 2008). We developed a strategy to identify such genes based on the notion that malignant cell transformation is a highly cooperative process (Hahn et al., 1999; Land et al., 1983) and involves synergistic regulation of non-mutant genes and proteins downstream of oncogenic mutations (Lloyd et al., 1997; Sewing et al., 1997; Xia and Land, 2007). In fact, we were able to demonstrate that genes regulated synergistically by multiple cancer gene mutations, i.e. ‘cooperation response genes’ (CRGs), are highly enriched for non-mutant drivers of the cancer phenotype (McMurray et al., 2008). Notably, CRGs contain a large fraction of genes involved in the control of cell metabolism (McMurray et al., 2008), thus pinpointing potential causal linkages between oncogenic mutations and the regulation of cancer cell metabolism.

The presence among CRGs of ATP-binding cassette transporter A1/cholesterol exporter (ABCA1) gene, a key player in cholesterol metabolism, prompted us to investigate the role of ABCA1 in regulating both intra-cellular cholesterol levels and the cancer phenotype. The ABCA1 protein mediates the transfer of cellular cholesterol across the plasma membrane to apolipoprotein A-I (ApoAI), the major apolipoprotein component of HDL (Attie, 2007). Loss-of-function mutations in the ABCA1 gene induce Tangier disease and familial hypoalphalipoproteinemia, both cardio-vascular conditions characterized by abnormally low levels of HDL-bound serum cholesterol and excess cholesterol in macrophages followed by vascular plaque (Singaraja et al., 2006). Based on our previous identification of ABCA1 as a synergistically down-regulated CRG (McMurray et al., 2008) and its well-characterized function as cholesterol exporter, we hypothesized that loss of ABCA1 efflux function

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

promotes carcinogenesis presumably via increasing intra-cellular cholesterol levels. Similarly, we wanted to explore whether somatic mutations in the ABCA1 gene found in human colon cancers following genomic DNA sequencing (Sjöblom et al., 2006) cause defects in ABCA1 efflux function and thus may facilitate malignant cell transformation.

Results

ABCA1-mediated tumor inhibition

Cooperation response genes (CRGs) were identified by virtue of their synergistic regulation by mutant $p53^{175H}$ (mp53) and activated H-Ras^{V12} (Ras) at the level of polysomal RNA abundance in young adult mouse colon (YAMC) cells (McMurray et al., 2008). The list of CRGs includes the gene encoding the cholesterol exporter ABCA1, which is synergistically down-regulated both at RNA (McMurray et al., 2008) and protein (Figure 1A) levels in cells expressing mp53 and Ras (mp53/Ras cells), as compared to cells expressing either mp53 or Ras alone.

Gene perturbation experiments indicate that ABCA1 has strong anti-tumor activity. Ectopic ABCA1 re-expression in mp53/Ras cells to levels approximating those found in the parental YAMC cells, i.e. ABCA1 reconstitution, (Figure 1B) caused a marked reduction in the size of tumors forming after implantation of the genetically modified cells into the flanks of immuno-compromised mice (Figure 1C). ABCA1-associated tumor inhibition is further indicated by the loss of ectopic ABCA1 expression in the resulting tumors. ABCA1 mRNA levels were found three-fold lower in tumor tissue than in cells prior to implantation (Figure S1A), presumably due to rapid selection from the retrovirally transduced polyclonal cell population expressing ectopic ABCA1 at various levels. Consistent with this interpretation, ectopic expression of genes not affecting tumor growth was observed to remain relatively stable following implantation into mice (example shown in Figure S1B and S1C). Taken together, these results indicate a causal role for ABCA1 loss-of-function in malignant cell transformation. As ABCA1-reconstituted cells were rapidly excluded from tumors, analysis of mechanisms underlying ABCA1-mediated tumor inhibition in tumor tissue was not feasible. We observed, however, that ABCA1-reconstituted mp53/Ras cells showed increased rates of non-apoptotic cell death/necrosis *in vitro*, as compared to unperturbed controls, when cells were cultivated at a low cell density and in the absence of serum and other survival factors (Figure 1D). Under similar conditions proliferation rates remained unaffected (data not shown). This suggests that loss of ABCA1 expression, as found in mp53/Ras cells contributes to the relative cell death resistance characteristic of cancer cells.

ABCA1 loss-of-function in cardiovascular pathogenesis is associated with diminished cholesterol export activity and thus increased intra-cellular cholesterol (Singaraja et al., 2006). A similar scenario may result from suppression of ABCA1 expression in malignant cell transformation induced by cooperating oncogenic mutations. In fact, perturbations of the cholesterol synthesis pathway mimic the effects of ABCA1 reconstitution. Several steps downstream of HMG-CoAR, in the cholesterol biosynthetic pathway, lanosterol cyclase (LC) produces the first sterol intermediate, and pharmacological inhibition of this enzyme has been demonstrated to block cholesterol synthesis (Dollis and Schuber, 1994). As we show here, both a pharmacological LC inhibitor, and genetic perturbation of LC protein via shRNA-mediated knock down (Figure S1D-S1F) induce cell death as observed for ABCA1 reconstitution (Figure 1D). Moreover, tumors arising after implantation of LC knock down cells into mice efficiently escape from LC knock down (Figure S1G), consistent with a tumor-inhibitory effect of LC knock down and presumably due to negative selection.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

ABCA1 lowers mitochondrial cholesterol and promotes cytochrome C release

Due to its role as a cholesterol exporter, reconstitution of ABCA1 expression in mp53/Ras cells up to levels found in untransformed parental YAMC cells (Fig. 1B) is predicted to induce depletion of cellular cholesterol stores. Increased cholesterol in cancer cells has been found specifically in mitochondria (Feo et al., 1973; Feo et al., 1975; Montero et al., 2008). This led us to measure cholesterol levels in mitochondrial fractions, where we observed significantly lower levels of cholesterol in samples from ABCA1-reconstituted mp53/Ras cells as compared to controls (Figure 2A and S2A). Comparison of cholesterol levels in total cell membranes, however, indicated no significant overall differences between ABCA1-reconstituted and control mp53/Ras cells (data not shown).

Elevated cholesterol levels as found in the membranes of cancer cell mitochondria decrease membrane fluidity (Colell et al., 2003; Montero et al., 2008). This in turn inhibits mitochondrial permeability transition (MPT) and the associated matrix swelling and release from mitochondria of cell death-promoting molecules such as cytochrome C (Colell et al., 2003; Montero et al., 2008), presumably supporting cancer cell survival. Conversely, lowering mitochondrial cholesterol in cancer cells, as in the case of increased ABCA1 expression, would be predicted to increase sensitivity to MPT in response to stress. We thus hypothesized that ABCA1-associated tumor inhibition may be linked to its ability to lower mitochondrial cholesterol. Consistent with this idea, mitochondria from ABCA1-reconstituted mp53/Ras cells (Figure 1B) released more cytochrome C (Figure 2C) and are more sensitive to matrix swelling (Figure 2E) in response to Ca^{2+} than mitochondria from controls. Notably, we also could show that the ABCA1-mediated sensitization of both cytochrome C release and matrix swelling is dependent on the associated decrease in mitochondrial cholesterol. This is demonstrated by the inhibition of ABCA1-induced cytochrome C release and matrix swelling, following restoration of cholesterol in isolated mitochondria to levels found in controls (Figure 2A, 2C, and 2E).

Independent support that lowering mitochondrial cholesterol is sufficient to enable mitochondrial cytochrome C release in response to stress comes from experiments targeting the cholesterol synthesis pathway via knock down of lanosterol cyclase (LC) in mp53/Ras cells (Figure S1F). This perturbation not only induces a reduction in mitochondrial cholesterol (Figure 2B and S2A), but also increased mitochondrial sensitivity to calcium induced cytochrome c release (Figure 2D) and matrix swelling (Figure 2F), to levels similar to those seen in ABCA1-reconstituted cells (Figure 2A, 2C, and 2E). Moreover, restoration of cholesterol in mitochondria from LC knock down cells reversed this sensitization (Figure 2B, 2D, and 2F). In this context it is relevant to mention that cell death was not detected in any cells used for preparation of mitochondria, as these cells were cultured at higher cell densities than cells used for measuring the impact of ABCA1 reconstitution and LC knock down on cell survival. Our data thus suggest reduction of mitochondrial cholesterol as a causal factor in ABCA1-mediated matrix swelling and cytochrome C release.

In addition to lowering mitochondrial cholesterol, ABCA1 has been associated with decreased lipid raft cholesterol (without detectable alterations in overall levels of plasma membrane cholesterol) and reduced Akt activity (Landry et al., 2006). A reduction in Akt-dependent survival signaling thus may also contribute to the anti-cancer activity of ABCA1. We found, however, no evidence for decreased AKT activity in response to ABCA1 reconstitution in mp53/Ras cells, as indicated by unaltered phosphorylation at AKT residues Ser473 and Thr308 (data not shown). This may be due to the presence of activated Ras in the mp53/Ras cells, a potent activator of AKT (Repasky et al., 2004). AKT inhibition thus appears unlikely to contribute to ABCA1-associated tumor inhibition in mp53/Ras cells. Moreover, in our system we were unable to confirm reports linking increased cholesterol to

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

elevated endoplasmic reticulum stress and the associated unfolded protein response (Tabas, 2010) (data not shown).

Our data described above indicate that mitochondrial cholesterol in cancerous cells is controlled effectively by both cholesterol biosynthesis and the cholesterol exporter ABCA1. Consistent with this idea, elevated mitochondrial cholesterol in mp53/Ras cells is accompanied not only by reduced ABCA1 expression (Figure 1A), but also by increased cholesterol synthesis rates, as compared to YAMC, Ras, or mp53 cells (Figure S2B). Correspondingly, levels of mitochondrial cholesterol in mp53/Ras cells are uniquely higher than in YAMC, Ras or mp53 cells (Figure S2C and S2D). As one might predict in such a context, decreasing ABCA1 expression in cells in which cholesterol synthesis is not fully activated, i.e. reduction of ABCA1 levels in Ras and mp53 cells to levels found in mp53/Ras cells (Figure S2E), did not increase mitochondrial cholesterol (Figure S2D), presumably because of insufficient supply. Moreover, mitochondrial cholesterol is also not increased by an additional reduction of ABCA1 expression in malignant mp53/Ras cells (Figure S2D), suggesting saturation of mitochondrial cholesterol in these cells. Notably, in either context tumor formation capacity was not affected (Figure S2F and S2G), presumably because mitochondrial cholesterol levels remained unaltered. Taken together, it thus seems reasonable to suggest that ABCA1 functions to restrict tumor growth via maintaining mitochondrial cholesterol at levels incompatible with the cancer phenotype, particularly in the presence of elevated rates of cholesterol synthesis that would otherwise drive up mitochondrial cholesterol.

Based on our data summarized above we hypothesized that elevated mitochondrial cholesterol can promote increased death resistance in cancer cells and thus malignant cell transformation. Such a view is supported by work of others showing that RNAi knockdown of squalene synthase, the enzyme catalyzing the first committed step of cholesterol synthesis, leads to decreased cell proliferation and survival of prostate cancer cells *in vitro* (Bresselmans et al., 2007), and that shRNA-mediated knockdown of the steroidogenic acute regulatory protein (StAR), a mitochondrial protein which transports cholesterol into mitochondria, sensitized hepatocellular carcinoma cells to chemotherapeutic agents *in vitro* (Montero et al., 2008). Nevertheless, direct demonstration of a causal role of metabolites, such as cholesterol, in intact cells cannot be achieved by genetic manipulation. We thus further examined the validity of our hypothesis by testing several independent predictions in a variety of biological contexts.

ABCA1 promotes mitochondria-mediated cell death in cancer cells

ABCA1 promotes reduction in mitochondrial cholesterol, MPT associated cytochrome C release, and non-apoptotic/necrotic cell death in response to cell stress, suggesting that ABCA1-mediated cell death depends on cholesterol for modulation of mitochondrial function. We tested this prediction in two steps, first by examining whether ABCA1-induced cell death requires MPT pore components and second by testing whether reduction of mitochondrial cholesterol levels by independent means can mimic the effects of ABCA1 re-expression. Mitochondria-dependent cell death occurs following MPT, during which mitochondrial proteins form membrane pores that enable the release of molecules such as cytochrome C, which can initiate both apoptotic and non-apoptotic cell death. (Kroemer et al., 2007; Nakagawa et al., 2005). Cyclophilin D (CypD) and the adenine nucleotide translocator (Ant1) are two mitochondrial proteins that play key roles in initiating MPT (Baines et al., 2005; Lee et al., 2009; Nakagawa et al., 2005). To test whether CypD or Ant1 are required for the increased death sensitivity of ABCA1-positive mp53/Ras cells and ABCA1 anti-cancer activity, we lowered expression of CypD or Ant1 via shRNA-mediated knockdown. We found that both CypD and Ant1 are essential for ABCA1-mediated death sensitization and tumor inhibition (Figure 3), suggesting that the anti-tumor activity of

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

ABCA1 requires mitochondrial function. In addition, reduction of mitochondrial cholesterol following knockdown of lanosterol cyclase (LC) in mp53/Ras cells not only induces cytochrome c release (Figure 2), but also facilitates cell death in an CypD and Ant1-dependent manner (Figure S3) indistinguishable from ABCA1-positive cells. Taken together, these data support the idea that ABCA1-induced reduction of mitochondrial cholesterol sensitizes cancer cells to stimuli or stresses promoting cell death.

Cholesterol efflux function linked to ABCA1 anti-tumor activity

Further evidence substantiating a causal link between ABCA1-mediated tumor inhibition and mitochondrial cholesterol depletion comes from testing the dependence of ABCA1 anti-cancer activity on its efflux capacity. To this end, we utilized two missense mutations of human ABCA1 proteins (Q597R and C1477R) linked to Tangier disease (TD) and familial hypoalphalipoproteinemia (FHA), which were previously characterized to have diminished cholesterol efflux activity (Singaraja et al., 2006). Functionality of these ABCA1 mutants in terms of tumor inhibition and cholesterol efflux was tested in human cancer cells lacking endogenous ABCA1 expression, as preliminary experiments revealed that human ABCA1 is inactive in murine cells, indicating a cross-species incompatibility (data not shown). We thus first confirmed that ectopically expressed wild type human ABCA1 inhibited tumor growth of HT-29 and DLD-1 human colon cancer cells when implanted subcutaneously into immuno-compromised mice and increased cholesterol efflux capacity in both cell types (Figure 4). In contrast, both TD/FHA mutants, although expressed to similar levels as wild type ABCA1, did not decrease tumor formation, and as expected were deficient for cholesterol efflux (Figure 4), thus supporting a causal link between ABCA1's anti-tumor activity and its efflux capacity.

Notably, somatic mutations in the ABCA1 gene have been discovered following genomic sequencing of human colon cancer samples. In addition to the well known cancer genes (i.e. p53, Apc and Ras), ABCA1 placed in the top group of genes mutated in human colon cancers at low frequency, with four separately occurring single base pair substitutions identified (Sjöblom et al., 2006). Given the correlation between tumor inhibition and efflux activity of ABCA1 described above, we sought to test whether the ABCA1 colon cancer mutations affect efflux and anti-tumor activity similar to the TD/FHA mutants. We thus engineered each of these four colon cancer associated mutations individually into a human ABCA1 cDNA to test their effects on ABCA1 functionality. Two of the mutants (A1407T and A2109T) demonstrated a marked reduction in both cholesterol efflux and anti-tumor activity, while the other two mutants (E210D and D917Y) showed activity indistinguishable from the wild type (wt) ABCA1 cDNA in both assays and in both human cancer cell lines used (Figure 4). Our data thus identify ABCA1 human colon cancer mutations A1407T and A2109T as loss-of-function mutations disabling both cholesterol efflux and anti-tumor activity.

Additional evidence to support the conclusion that efflux-deficient ABCA1 mutants lose anti-tumor activity is provided by ABCA1 expression analysis in tissue samples pre and post implantation into mice. While expression of wt ABCA1 is strongly reduced following transplantation (see also Figure S1A for murine ABCA1), expression of loss-of-function ABCA1 mutants A1407T and A2109T remains relatively unchanged, presumably because their anti-tumor activity is strongly diminished (Figure S4).

In summary, our data reveal a close correlation between cholesterol efflux capacity, reduction of mitochondrial cholesterol in presence of elevated cholesterol synthesis rates and anti-tumor activity of ABCA1. We also demonstrate a loss-of-function phenotype for somatic mutations identified in human colon cancer and thus suggest a role of ABCA1 as a

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

tumor suppressor, albeit specifically in the context of increased cholesterol synthesis rates competent to raise levels of mitochondrial cholesterol.

Discussion

Here we demonstrate an anti-cancer function of the cholesterol exporter ABCA1 in human cancer cells. Our data link malignant cell transformation to defective cholesterol efflux, either following suppression of ABCA1 gene expression in response to cooperating oncogenic mutations or loss-of-function mutation. ABCA1 deficiency allows for elevated mitochondrial cholesterol and ultimately supports cancer cell survival. We suggest that this involves increased retention of cell death-promoting molecules in mitochondria, presumably due to decreased membrane fluidity and inhibition of mitochondrial permeability transition (MPT) (Colell et al., 2003; Montero et al., 2008). Conversely, ABCA1 anti-tumor activity requires efflux function and appears to be mediated by reduced mitochondrial cholesterol combined with an increased potential for release of cell death promoting molecules, such as cytochrome C, from mitochondria.

ABCA1 loss-of-function mutations play a causal role in Tangier disease (TD) and familial hypoalphalipoproteinemia (FHA), cardiovascular conditions characterized by abnormally low levels of plasma HDL-cholesterol (HDL-C) (Rust et al., 1999). Missense mutations identified in the ABCA1 gene of TD/FHA patients reduce efflux function, leading to a diminished ability of ABCA1 to export excess intracellular cholesterol to apolipoprotein A1, forming HDL-C particles (Singaraja et al., 2006). While TD/FHA has not been linked to cancer, possibly due to the relatively low incidence of TD/FHA, low serum HDL levels, a characteristic feature of TD/FHA, correlate with higher incidence of multiple cancers (Liu et al., 2011; McGrowder et al., 2011; Van Hemelrijck et al., 2011). Our finding that multiple independent ABCA1 missense mutations found in TD/FHA patients and human colon cancers show loss of both cholesterol export function and anti-tumor activity, supports an anti-cancer function of ABCA1 in human malignancy. In addition our data strongly argue in favor of a causal link between export and anti-cancer functions.

Genome-scale DNA sequencing of human cancer samples has revealed novel tumor associated genetic alterations, thus identifying numerous candidate oncogenes or tumor suppressor genes (Beroukhim et al., 2010; C.G.A.R.N., 2008; Ding et al., 2008; Sjöblom et al., 2006). Specifically, sequencing DNA from human colon cancer specimen identified, among others, four independently occurring somatic mutations in the ABCA1 gene, albeit with lower frequently than the well known oncogenic mutations in genes such as Apc, p53, and Ras (Sjöblom et al., 2006). Here we demonstrate that two of four mutations identified in colon cancer samples are loss-of-function mutations. The two other ABCA1 mutations, in contrast, had no significant effects on ABCA1 mediated efflux or tumor inhibition, and thus may represent functionally neutral passenger mutations serendipitously co-selected with other cancer-relevant driver mutations (Forrest and Cavet, 2007; Getz et al., 2007; Parmigiani et al., 2007; Rubin and Green, 2007). Alternatively, these mutations may exhibit a subtle and rather context-dependent loss-of-function phenotype, e.g. dependence on inter-molecular interactions, that remains undetected in our experimental models.

Diminished cholesterol efflux can be presumed a likely consequence of somatic ABCA1 loss-of-function mutations in human colon cancers, although the mutations were found to be heterozygous (Sjöblom et al., 2006). While TD/FHA patients exhibiting almost complete absence of plasma HDL-C are typically homozygous for ABCA1 loss-of-function mutations, heterozygosity of ABCA1 is sufficient to produce intermediate HDL-C deficiencies (Singaraja et al., 2006). This suggests haplo-insufficiency for ABCA1 function, thus rendering cholesterol export particularly vulnerable not only to gene mutation but also

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

to partial suppression of ABCA1 gene expression, particularly in scenarios where this will lead to increased mitochondrial cholesterol. In fact, inhibition of ABCA1 expression has been observed in human colon, breast, lymphoblastic and liver cancer specimen (Basso et al., 2005; Ki et al., 2007; Moustafa et al., 2004; Schimanski et al., 2009), indicating decreased ABCA1 efflux activity in a variety of human cancers.

In addition to loss of cholesterol export activity, ABCA1 mutations linked to TD/FHA, including those utilized in the current study, also show deficiency in phosholipid efflux (Singaraja et al., 2006). As a key component of HDL particles, phospholipids play an important role in the removal of cellular cholesterol (Attie, 2007) and thus diminished phospholipid efflux may exacerbate defects in cholesterol efflux. Although we cannot exclude the possibility that ABCA1 loss-of-function effects are in part mediated by metabolites such as phospholipids, extensive experimentation described above indicates that lowering cholesterol synthesis closely mimics the effects of ABCA1 suppression or loss-of-function, thus suggesting cholesterol as a key driver of the observed effects.

Cholesterol lowering drugs, such as statins, in use for their benefits in cardiovascular disease have been evaluated for their efficacy in prevention and treatment of cancer. A variety of retrospective studies have revealed association between long term statin use and lower cancer incidence (Cauley et al., 2003; Farwell et al., 2008; Karp et al., 2008; Khurana et al., 2007; Poynter et al., 2005; Shannon et al., 2005), as well as improved outcome (Gutt et al., 2010; Kawata et al., 2001; Nowakowski et al., 2010; Siddiqui et al., 2009). In addition, when combined with chemotherapy, statins significantly augment efficacy of therapy in patients with acute myelogenous leukemia (Kornblau et al., 2007), while little to no anti-cancer effects of statins have been observed when used in patients as single agents (Holstein et al., 2006; Knox et al., 2005; Thibault et al., 1996). Therapeutic efficacy of statins in combination with chemotherapy may be due to the capability of statins to sensitize cancer cells to chemotherapy-induced cell death (Kornblau et al., 2007). This chemo-sensitization is consistent with the anti-tumor mechanism of lowering cholesterol proposed here and by others (Montero et al., 2008), where decreased cholesterol in mitochondria leads to increased sensitivity to stimuli inducing cell death, presumably by facilitating MPT.

The in vivo genetic evidence provided here reveals anti-cancer activity of the cholesterol exporter ABCA1, which can be disabled by ABCA1 loss-of-function mutations or suppression of ABCA1 gene expression in response to cooperating oncogenic mutations. Moreover, we suggest that loss of ABCA1-mediated efflux is a key step in allowing the accumulation of mitochondrial cholesterol levels that support cancer survival. Taken together, our data thus support a causal role for elevated mitochondrial cholesterol in cancer cells and provide a mechanistic rationale for cholesterol targeting as a potentially fruitful approach to augment cancer intervention and/or prevention strategies.

Materials and Methods

Cells

Young adult mouse colon (YAMC) cells (Whitehead et al., 1993), and YAMC cells expressing $p53^{175H}$ (mp53), $HRas^{V12}$ (Ras) and both $p53^{175H}$ and $HRas^{V12}$ (mp53/Ras) cells were maintained as previously described (McMurray et al., 2008; Xia and Land, 2007). DLD-1 human colon cancer cells were provided by Dr. J. Filmus and HT-29 human colon cancer cells were purchased from ATCC. Both human cell lines were cultured at 37°C in DMEM media (Invitrogen) supplem ented with 10% FBS (Hyclone) and 2.5μg/mL genamycin (Invitrogen). See supplementary methods for derivation of genetically perturbed cells.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Xenograft Assay

Tumor formation was assayed as previously described (McMurray et al., 2008). See supplementary methods for detailed description.

Cell Death Assays

Prior to cell death assay, mp53/Ras cells were grown at 39°C for at least 48 hr. To assess resistance to cell death, mp53/Ras cells were seeded at low density (2265 cells/cm^2) in standard RPMI growth media. Sixteen hr later, cells were washed with PBS and then given RPMI media devoid of serum and ITS. Media given to ABCA1-reconstituted and matched vector control mp53/Ras cells was also supplemented with 20μg/ml apolipoprotein A1 (Meridian Life Science) to lower intracellular cholesterol by inducing cholesterol efflux. These cells were given a second dose of 20μg/ml apolipoprotein 48 hr later. Cell death was measured 72 hr after initial starvation by trypan blue exclusion assay as described in product manual (Invitrogen), or fixed in 4% paraformaldehye and TUNEL stained (Roche). The amount of TUNEL positive cells was quantified via fluorescence-activated cell sorting (FACS) analysis.

Intracellular Cholesterol Measurements

Prior to measuring intracellular cholesterol, cells were grown at 39°C for at least 48 hr followed by growth in RPMI media, devoid of serum and ITS, for 72 hr. Media given to ABCA1-reconstituted, ABCA1-knockdown, and matched vector control cells during this 72 hour time period was also supplemented with 20μg/ml apolipoprotein A1 to induce cholesterol efflux. A second dose of 20μg/ml apolipoprotein A1 was given after 48 hr. Intracellular cholesterol levels were determined using the Amplex Red Cholesterol Assay Kit (Invitrogen) or thin layer chromatography. See supplemental methods for more detailed description of cholesterol measurements.

Mitochondrial Cholesterol Loading, Matrix Swelling, and Cytochrome C Release Assays

Cholesterol levels were restored in cholesterol-depleted mitochondria of ABCA1-reconstituted and lanosterol cyclase-knockdown mp53/Ras cells as described previously (Montero et al., 2008). Sensitivity to calcium induced mitochondrial matrix swelling and cytochrome c release was then determined as previously described (Montero et al., 2008).

Cholesterol Efflux Assay

Cholesterol efflux was measured as previously described (Singaraja et al., 2006). Briefly, cells were given 1μCi/mL [H^3]cholesterol (Perkin-Elmer) for 16 hr, followed by washing with PBS containing 0.2% fatty acid free BSA (Sigma). Cells were then incubated for 24 hr in serum free DMEM containing 0.2% fatty acid free BSA and 20 μg/mL Apolipoprotein A1. Following incubation, media containing effluxed cholesterol was collected and centrifuged at 10,000xg for 5 min to remove debris. Radioactivity was quantified by liquid scintillation and normalized to total protein. Efflux rate was calculated as a percentage of efflux by wild-type ABCA1 and indicated values represent the average of three independent experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Acknowledgments

We would like to thank Dr. D. Brdiczka for critical advice and discussion, Dr. H. McMurray for helpful suggestions and Drs. A.R. Tall, M.R. Hayden and J. Filmus for reagents. This work was supported by NIH grants CA90663, CA120317 and CA138249.

References

Attie AD. ABCA1: at the nexus of cholesterol, HDL and atherosclerosis. Trends Biochem Sci. 2007; 32:172–179. [PubMed: 17324574]

Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, Brunskill EW, Sayen MR, Gottlieb RA, Dorn GW, et al. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. Nature. 2005; 434:658–662. [PubMed: 15800627]

Basso K, Margolin AA, Stolovitzky G, Klein U, Dalla-Favera R, Califano A. Reverse engineering of regulatory networks in human B cells. Nature genetics. 2005; 37:382–390. [PubMed: 15778709]

Benn M, Tybjarg-Hansen A, Stender S, Frikke-Schmidt R, Nordestgaard B. Low-Density Lipoprotein Cholesterol and the Risk of Cancer: A Mendelian Randomization Study. JNCI. 2011; 103:1–12.

Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, Barretina J, Boehm JS, Dobson J, Urashima M, et al. The landscape of somatic copy-number alteration across human cancers. Nature. 2010; 463:899–905. [PubMed: 20164920]

Bresselmans K, Timmermans L, Van de Sande T, Veldhoven P, Guan G, Shechter I, Claessens F, Verhoeven G, Swinnen J. Squalene Synthase: a determinant of raft-associated cholesterol and modulator of cancer cell proliferation. JBC. 2007; 282:18777–18785.

C.G.A.R.N. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008; 455:1061–1068. [PubMed: 18772890]

Caruso MG, Notarnicola M, Cavallini A, Di Leo A. 3-Hydroxy-3-methylglutaryl coenzyme A reductase activity and low-density lipoprotein receptor expression in diffuse-type and intestinal-type human gastric cancer. J Gastroenterol. 2002; 37:504–508. [PubMed: 12162407]

Caruso MG, Notarnicola M, Santillo M, Cavallini A, Di Leo A. Enhanced 3-hydroxy-3-methyl-glutaryl coenzyme A reductase activity in human colorectal cancer not expressing low density lipoprotein receptor. Anticancer Res. 1999; 19:451–454. [PubMed: 10226581]

Cauley JA, Zmuda JM, Lui LY, Hillier TA, Ness RB, Stone KL, Cummings SR, Bauer DC. Lipid-lowering drug use and breast cancer in older women: a prospective study. J Womens Health. 2003; 12:749–756.

Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, Fleming MD, Schreiber SL, Cantley LC. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nauture. 2008; 452:230–233.

Chuu CP, Hiipakka RA, Kokontis JM, Fukuchi J, Chen RY, Liao S. Inhibition of tumor growth and progression of LNCaP prostate cancer cells in athymic mice by androgen and liver X receptor agonist. Cancer Res. 2006; 66:6482–6486. [PubMed: 16818617]

Chuu CP, Lin HP. Antiproliferative effect of LXR agonists T0901317 and 22(R)-hydroxycholesterol on multiple human cancer cell lines. Anticancer Res. 2010; 30:3643–3648. [PubMed: 20944148]

Colell A, Garcia-Ruiz C, Lluis J, Coll O, Mari M, Fernandez-Checa J. Cholesterol Impairs the Adenine Nucleotide Translocator-mediated Mitochondrial Permeability Transition through Altered Membrane Fluidity. JBC. 2003; 278:33928–33935.

Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. Nat Rev Cancer. 2005; 5:930–942. [PubMed: 16341084]

Dessi S, Batetta B, Anchisi C, Pani P, Costelli P, Tessitore L, Baccino FM. Cholesterol metabolism during the growth of a rat ascites hepatoma. Br J Cancer. 1992; 66:787–793. [PubMed: 1419621]

Dessi S, Batetta B, Pulisci D, Spano O, Anchisi C, Tessitore L, Costelli P, Baccino FM, Aroasio E, Pani P. Cholesterol content in tumor tissues is inversely associated with high-density lipoprotein cholesterol in serum in patients with gastrointestinal cancer. Cancer. 1994; 73:253–258. [PubMed: 8293385]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, Sougnez C, Greulich H, Muzny DM, Morgan MB, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 2008; 455:1069–1075. [PubMed: 18948947]

Dollis D, Schuber F. Effects of a 2,3-oxidosqualene-lanosterol cyclase inhibitor 2,3:22,23-dioxidosqualene and 24,25-epoxycholesterol on the regulation of cholesterol biosynthesis in human hepatoma cell line HepG2. Biochem Pharmacol. 1994; 48:49–57. [PubMed: 8043030]

Fantin VR, St-Pierre J, Leder P. Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell. 2006; 9:425–434. [PubMed: 16766262]

Farwell WR, Scranton RE, Lawler EV, Lew RA, Brophy MT, Fiore LD, Gaziano JM. The association between statins and cancer incidence in a veterans population. J Natl Cancer Inst. 2008; 100:134–139. [PubMed: 18182618]

Feo F, Canuto RA, Bertone G, Garcea R, Pani P. Cholesterol and phospholipid composition of mitochondria and microsomes isolated from morris hepatoma. FEBS Lett. 1973; 33:229–232. [PubMed: 4354064]

Feo F, Canuto RA, Garcea R, Gabriel L. Effect of cholesterol content on some physical and functional properties of mitochondria isolated from adult rat liver, fetal liver, cholesterol-enriched liver and hepatomas AH-130, 3924A and 5123. Biochim Biophys Acta. 1975; 413:116–134. [PubMed: 172147]

Forrest WF, Cavet G. Comment on “The Consensus Coding Sequences of Human Breast and Colorectal Cancers”. Science. 2007; 317:1500. [PubMed: 17872427]

Gao J, Jia WD, Li JS, Wang W, Xu GL, Ma JL, Ge YS, Yu JH, Ren WH, Liu WB, et al. Combined inhibitory effects of celecoxib and fluvastatin on the growth of human hepatocellular carcinoma xenografts in nude mice. J Int Med Res. 2010; 38

Getz G, Hofling H, Mesirov JP, Golub TR, Meyerson M, Tibshirani R, Lander ES. Comment on “The Consensus Coding Sequences of Human Breast and Colorectal Cancers”. Science. 2007; 317:1500. [PubMed: 17872428]

Graziani SR, Igreja FA, Hegg R, Meneghetti C, Brandizzi L, Barboza R, Amancio RF, Pinotti JA, Maranhao RC. Uptake of a cholesterol-rich emulsion by breast cancer. Gynecol Oncol. 2002; 85:493–497. [PubMed: 12051880]

Gregg RG, Davidson M, Wilce PA. Cholesterol synthesis and HMG CoA reductase activity during hepatocarcinogenesis in rats. International Journal of Biochemistry. 1986; 18:389–393. [PubMed: 2872090]

Gutt R, Tonlaar N, Kunnavakkam R, Karrison T, Weichselbaum RR, Liauw SL. Statin use and risk of prostate cancer recurrence in men treated with radiation therapy. J Clin Oncol. 2010; 28:2653–2659. [PubMed: 20421534]

Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. Creation of human tumour cells with defined genetic elements. Nature. 1999; 400:464–468. [PubMed: 10440377]

Hentosh P, Yuh SH, Elson CE, Peffley DM. Sterol-independent regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in tumor cells. Molecular Carcinogenesis. 2001; 32:154–166. [PubMed: 11746827]

Holstein SA, Knapp HR, Clamon GH, Murry DJ, Hohl RJ. Pharmacodynamic effects of high dose lovastatin in subjects with advanced malignancies. Cancer Chemother Pharmacol. 2006; 57:155–164. [PubMed: 16133537]

Huang EH, Johnson LA, Eaton K, Hynes MJ, Carpentino JE, Higgins PD. Atorvastatin induces apoptosis in vitro and slows growth of tumor xenografts but not polyp formation in MIN mice. Dig Dis Sci. 2010; 55:3086–3094. [PubMed: 20186482]

Jacobs D, Blackburn H, Higgins M, Reed D, Iso H, McMillan G, Neaton J, Nelson J, Potter J, Rifkind B. Report of the conference on low blood cholesterol: mortality associations. Circulation. 1992; 86:1046–1060. [PubMed: 1355411]

Karp I, Behlouli H, Lelorier J, Pilote L. Statins and cancer risk. Am J Med. 2008; 121:302–309. [PubMed: 18374689]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Kawata S, Yamasaki E, Nagase T, Inui Y, Ito N, Matsuda Y, Inada M, Tamura S, Noda S, Imai Y. Effect of pravastatin on survival in patients with advanced hepatocellular carcinoma. A randomized controlled trial. Br J Cancer. 2001; 84:886–891. [PubMed: 11286466]

Khurana V, Bejjanki HR, Caldito G, Owens MW. Statins reduce the risk of lung cancer in humans: a large case-control study of US veterans. Chest. 2007; 131:1282–1288. [PubMed: 17494779]

Ki DH, Jeung HC, Park CH, Kang SH, Lee GY, Lee WS, Kim NK, Chung HC, Rha SY. Whole genome analysis for liver metastasis gene signatures in colorectal cancer. Int J Cancer. 2007; 121:2005–2012. [PubMed: 17640062]

Knox JJ, Siu LL, Chen E, Dimitroulakos J, Kamel-Reid S, Moore MJ, Chin S, Irish J, LaFramboise S, Oza AM. A Phase I trial of prolonged administration of lovastatin in patients with recurrent or metastatic squamous cell carcinoma of the head and neck or of the cervix. Eur J Cancer. 2005; 41:523–530. [PubMed: 15737556]

Kochuparambil ST, Al-Husein B, Goc A, Soliman S, Somanath PR. Anticancer efficacy of simvastatin on prostate cancer cells and tumor xenografts is associated with inhibition of Akt and reduced prostate-specific antigen expression. J Pharmacol Exp Ther. 2011; 336:496–505. [PubMed: 21059805]

Kolanjiappan K, Ramachandran CR, Manoharan S. Biochemical changes in tumor tissues of oral cancer patients. Clin Biochem. 2003; 36:61–65. [PubMed: 12554062]

Kornblau SM, Banker DE, Stirewalt D, Shen D, Lemker E, Verstovsek S, Estrov Z, Faderl S, Cortes J, Beran M. Blockade of adaptive defensive changes in cholesterol uptake and synthesis in AML by the addition of pravastatin to idarubicin+high-dose Ara-C: a phase 1 study. Blood. 2007; 109:2999–3006. [PubMed: 17158228]

Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. Physiol Rev. 2007; 87:99–163. [PubMed: 17237344]

Land H, Parada LF, Weinberg RA. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature. 1983; 304:596–602. [PubMed: 6308472]

Landry YD, Denis M, Nandi S, Bell S, Vaughan AM, Zha X. ATP-binding cassette transporter A1 expression disrupts raft membrane microdomains through its ATPase-related functions. J Biol Chem. 2006; 281:36091–36101. [PubMed: 16984907]

Lee J, Schriner SE, Wallace DC. Adenine nucleotide translocator 1 deficiency increases resistance of mouse brain and neurons to excitotoxic insults. Biochim Biophys Acta. 2009; 1787:364–370. [PubMed: 19366611]

Liu YL, Qian HX, Qin L, Zhou XJ, Zhang B. Serum LDL-C and LDL-C/HDL-C ratio are positively correlated to lymph node stages in males with colorectal cancer. Hepatogastroenterology. 2011; 58:383–387. [PubMed: 21661400]

Lloyd AC, Obermuller F, Staddon S, Barth CF, McMahon M, Land H. Cooperating oncogenes converge to regulate cyclin/cdk complexes. Genes Dev. 1997; 11:663–677. [PubMed: 9119230]

Luo J, Emanuele MJ, Li D, Creighton CJ, Schlabach MR, Westbrook TF, Wong KK, Elledge SJ. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell. 2009; 137:835–848. [PubMed: 19490893]

McGrowder D, Riley C, Morrison EY, Gordon L. The role of high-density lipoproteins in reducing the risk of vascular diseases, neurogenerative disorders, and cancer. Cholesterol. 2011; 2011:1–9.

McMurray HR, Sampson ER, Compitello G, Kinsey C, Newman L, Smith B, Chen SR, Klebanov L, Salzman P, Yakovlev A, et al. Synergistic response to oncogenic mutations defines gene class critical to cancer phenotype. Nature. 2008; 453:1112–1116. [PubMed: 18500333]

Montero J, Morales A, Llacuna L, Lluis J, Terrones O, Basanez G, Antonsson B, Prieto J, Garcia-Ruiz C, Colell A, et al. Mitochondrial Cholesterol Contributes to Chemotherapy Resistance in Hepatocellular Carcinoma. Cancer Res. 2008; 68:5246–5256. [PubMed: 18593925]

Moustafa MA, Ogino D, Nishimura M, Ueda N, Naito S, Furukawa M, Uchida T, Ikai I, Sawada H, Fukumoto M. Comparative analysis of ATP-binding cassette (ABC) transporter gene expression levels in peripheral blood leukocytes and in liver with hepatocellular carcinoma. Cancer Sci. 2004; 95:530–536. [PubMed: 15182435]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H, Inohara H, Kubo T, Tsujimoto Y. Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. Nature. 2005; 434:652–658. [PubMed: 15800626]

Notarnicola M, Messa C, Pricci M, Guerra V, Altomare DF, Montemurro S, Caruso MG. Up-regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in left-sided human colon cancer. Anticancer Research. 2004; 24:3837–3842. [PubMed: 15736419]

Nowakowski GS, Maurer MJ, Habermann TM, Ansell SM, Macon WR, Ristow KM, Allmer C, Slager SL, Witzig TE, Cerhan JR. Statin use and prognosis in patients with diffuse large B-cell lymphomaand follicular lymphoma in the rituximab era. J Clin Oncol. 2010; 28:412–417. [PubMed: 20008638]

Parmigiani G, Lin J, Boca JS, Sjoblom T, Jones S, Wood LD, Parsons DW, Barber T, Buckhaults P, Sanford DM, et al. Response to Comments on “The Consensus Coding Sequences of Human Breast and Colorectal Cancers”. Science. 2007; 317:1500.

Pickeral OK, Li JZ, Barrow I, Boguski MS, Makałowski W, Zhang J. Classical Oncogenes and Tumor Suppressor Genes: A Comparative Genomics Perspective. Neoplasia. 2000; 2:280–286. [PubMed: 10935514]

Poynter JN, Gruber SB, Higgins PD, Almog R, Bonner JD, Rennert HS, Low M, Greenson JK, Rennert G. Statins and the risk of colorectal cancer. N Engl J Med. 2005; 352:2184–2192. [PubMed: 15917383]

Repasky GA, Chenette EJ, Der CJ. Renewing the conspiracy theory debate: does Raf function alone to mediate Ras oncogenesis? Trends Cell Biol. 2004; 14:639–647. [PubMed: 15519853]

Robertson TB, Burnett TC. The influence of lecithin and cholesterin upon the growth of tumours. Jour Exper Med. 1913; xvii:344–352. [PubMed: 19867647]

Rubin AF, Green P. Comment on “The Consensus Coding Sequences of Human Breast and Colorectal Cancers”. Science. 2007; 317:1500. [PubMed: 17872429]

Rudling M, Collins VP. Low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA levels are coordinately reduced in human renal cell carcinoma. Biochim Biophys Acta. 1996; 1299:75–79. [PubMed: 8555255]

Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denefle P, et al. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet. 1999; 22:352–355. [PubMed: 10431238]

Schaffner CP. Prostatic cholesterol metabolism: Regulation and alteration. The prostatic cell: Structure and function. 1981:279–324.

Schimanski S, Wild PJ, Treeck O, Horn F, Sigruener A, Rudolph C, Blaszyk H, Klinkhammer-Schalke M, Ortmann O, Hartmann A, et al. Expression of the lipid transporters ABCA3 and ABCA1 is diminished in human breast cancer tissue. Horm Metab Res. 2009; 42:102–109. [PubMed: 19902402]

Schlabach MR, Luo J, Solimini NL, Hu G, Xu Q, Li MZ, Zhao Z, Smogorzewska A, Sowa ME, Ang XL, et al. Cancer proliferation gene discovery through functional genomics. Science. 2008; 319:620–624. [PubMed: 18239126]

Sewing A, Wiseman B, Lloyd AC, Land H. High-intensity Raf signal causes cell cycle arrest mediated by p21Cip1. Mol Cell Biol. 1997; 17:5588–5597. [PubMed: 9271434]

Shaffer AL, Emre NC, Lamy L, Ngo VN, Wright G, Xiao W, Powell J, Dave S, Yu X, Zhao H, et al. IRF4 addiction in multiple myeloma. Nature. 2008; 454:226–231. [PubMed: 18568025]

Shannon J, Tewoderos S, Garzotto M, Beer TM, Derenick R, Palma A, Farris PE. Statins and prostate cancer risk: A case-control study. Am J Epidemiol. 2005; 162:318–325. [PubMed: 16014776]

Siddiqui AA, Nazario H, Mahgoub A, Patel M, Cipher D, Spechler SJ. For patients with colorectal cancer, the long-term use of statins is associated with better clinical outcomes. Dig Dis Sci. 2009; 54:1307–1311. [PubMed: 19337834]

Singaraja RR, Visscher H, James ER, Chroni A, Coutinho JM, Brunham LR, Kang MH, Zannis VI, Chimini G, Hayden MR. Specific mutations in ABCA1 have discrete effects on ABCA1 function and lipid phenotypes both in vivo and in vitro. Circ Res. 2006; 99:389–397. [PubMed: 16873719]

Siperstein MD. Cholesterol, cholesterogenesis and cancer. Adv Exp Med Biol. 1995; 369:155–166. [PubMed: 7598003]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, et al. The consensus coding sequences of human breast and colorectal cancers. Science. 2006; 314:268–274. [PubMed: 16959974]

Strasak AM, Pfeiffer RM, Brant LJ, Rapp K, Hilbe W, Oberaigner W, Lang S, Borena W, Concin H, Diem G, et al. Time-dependent association of total serum cholesterol and cancer incidence in a cohort of 172,210 men and women: a prospective 19-year follow-up study. Ann Oncol. 2009; 20:1113–1120. [PubMed: 19164459]

Tabas I. The role of endoplasmic reticulum stress in the progression of atherosclerosis. Circ Res. 2010; 7:839–850. [PubMed: 20884885]

Tatidis L, Masquelier M, Vitols S. Elevated uptake of low density lipoprotein by drug resistant human leukemic cell lines. Biochem Pharmacol. 2002; 63:2169–2180. [PubMed: 12110376]

Thibault A, Samid D, Tompkins AC, Figg WD, Cooper MR, Hohl RJ, Trepel J, Liang B, Patronas N, Venzon DJ, et al. Phase I study of lovastatin, an inhibitor of the mevalonate pathway, in patients with cancer. Clin Cancer Res. 1996; 2:483–491. [PubMed: 9816194]

Van Hemelrijck M, Walldius G, Jungner I, Hammar N, Garmo H, Binda E, Hayday A, Lambe M, Holmberg L. Low levels of apolipoprotein A-I and HDL are associated with risk of prostate cancer in the Swedish AMORIS study. Cancer Causes Control. 2011; 22:1011–1019. [PubMed: 21562751]

Warburg O. The metabolism of tumors. 1930

Weinberg F, Hamanaka R, Wheaton WW, Weinberg S, Joseph J, Lopez M, Kalyanaraman B, Mutlu GM, Budinger GR, Chandel NS. Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. Proc Natl Acad Sci USA. 2010; 107:8788–8793. [PubMed: 20421486]

Whitehead RH, VanEeden PE, Noble MD, Ataliotis P, Jat PS. Establishment of conditionally immortalized epithelial cell lines from both colon and small intestine of adult H-2Kb-tsA58 transgenic mice. Proc Natl Acad Sci U S A. 1993; 90:587–591. [PubMed: 7678459]

Xia M, Land H. Tumor suppressor p53 restricts Ras stimulation of RhoA and cancer cell motility. Nat Struct Mol Biol. 2007; 14:215–223. [PubMed: 17310253]

Yoshioka Y, Sasaki J, Yamamoto M, Saitoh K, Nakaya S, Kubokawa M. Quantitation by (1)H-NMR of dolichol, cholesterol and choline-containing lipids in extracts of normal and phathological thyroid tissue. NMR Biomed. 2000; 13:377–383. [PubMed: 11114060]

Zender L, Xue W, Zuber J, Semighini CP, Krasnitz A, Ma B, Zender P, Kubicka S, Luk JM, Schirmacher P, et al. An oncogenomics-based in vivo RNAi screen identifies tumor suppressors in liver cancer. Cell. 2008; 135:852–864. [PubMed: 19012953]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Cholesterol export deficiency plays role in both cardio-vascular disease and cancer

Human colon cancer mutations in ABCA1gene disable cholesterol export function

Excess cholesterol in mitochondria arrests suicide mechanism in cancer cells

Release of cell death promoters from mitochondria is blocked by high cholesterol

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

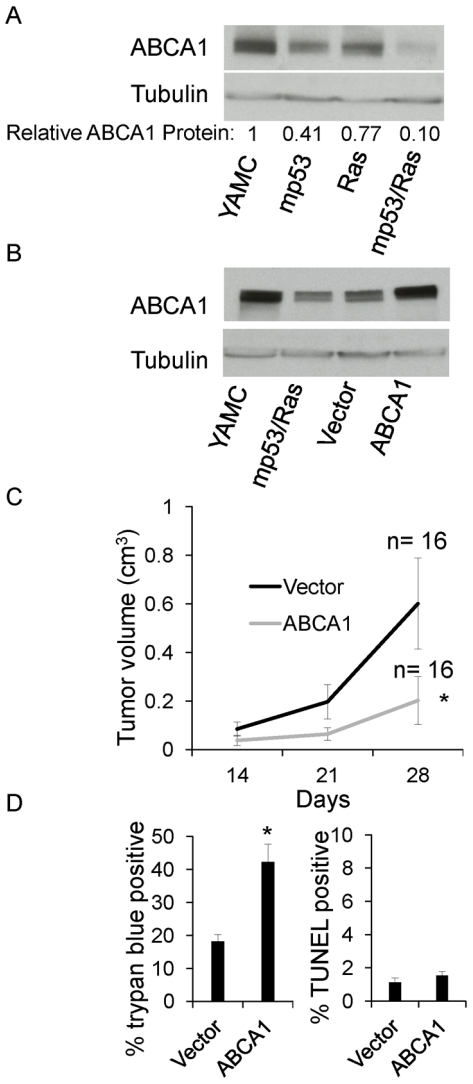

Figure 1. Reconstitution of ABCA1 Expression Inhibits Tumor Formation

(A) Western blot showing murine ABCA1 protein expression in YAMC, mp53, Ras and mp53/Ras cells.

(B) Western Blot showing stable re-expression of ectopic murine ABCA1 in mp53/Ras cells to levels found in YAMC parental cells. Tubulin served as loading control for western blots.

(C) Vector control or ABCA1-reconstituted mp53/Ras cells were subcutaneously injected in the flanks of nude mice and tumor volumes were measured at indicated times after injection. Values represent average of 16 tumors and error bars indicate standard deviation at each time point. *P <0.001, by student's t-test.

(D) Cell death of vector control and ABCA1-reconstituted mp53/Ras cells (ABCA1). Cell death was measured by trypan blue exclusion (left panel), with no indication for DNA fragmentation/apoptosis, as measured by TUNEL staining (right panel). Values represent the average of three independent experiments. Error bars indicate standard deviation. *P <0.01 versus vector control, by student's t-test.

See also Figure S1

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

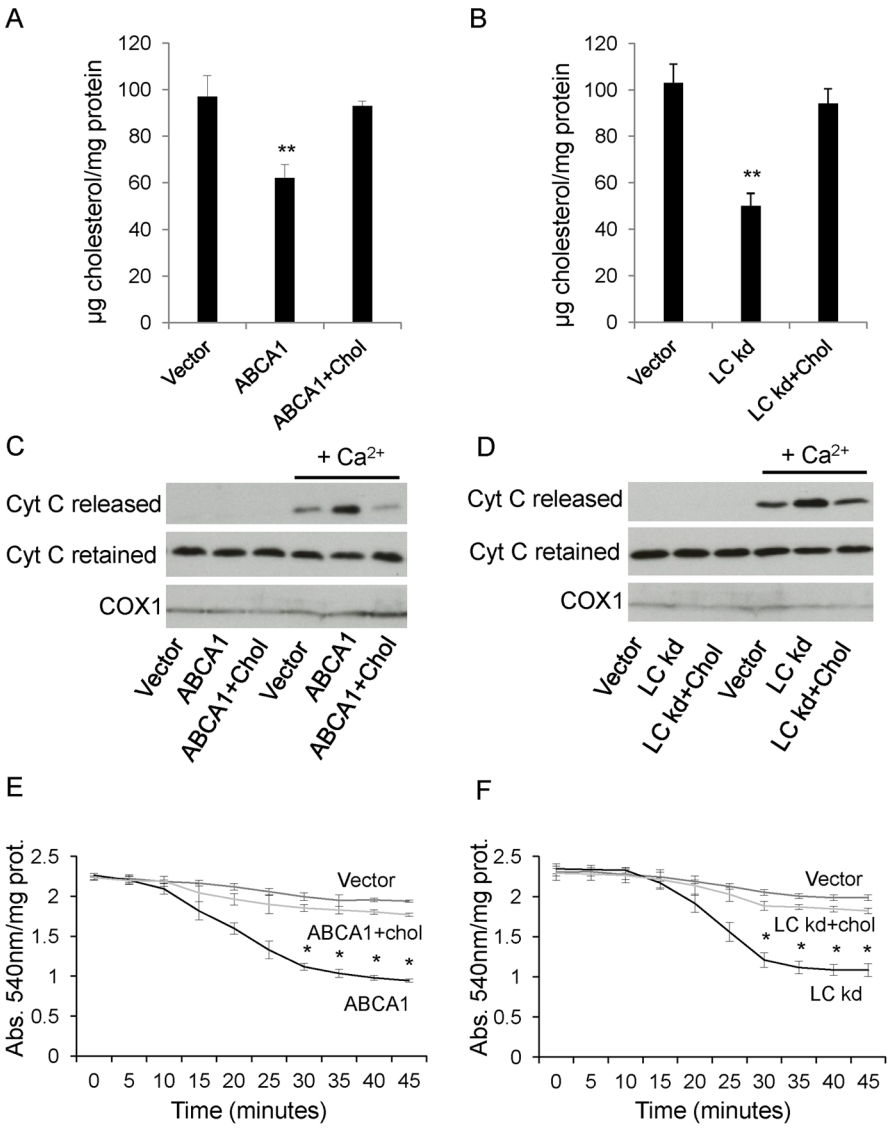

Figure 2. ABCA1 Reconstitution and Knockdown of Lanosterol Cyclase (LC) Decrease Mitochondrial Cholesterol and Increase Sensitivity to Calcium-Induced Cytochrome C Release and Matrix Swelling

(A, B) Cholesterol in mitochondria from vector control, ABCA1-reconstituted (ABCA1), and lanosterol cyclase knockdown (LC kd) mp53/Ras cells and following cholesterol restoration (ABCA1+Chol and LC kd+Chol). Cholesterol levels determined by Amplex Red cholesterol assay and normalized to total mitochondrial protein. Indicated values represent average from three independent experiments and (B) two independent LC shRNA constructs. Error bars indicate standard deviation, **P < 0.01 versus vector by student's t-test.

(C, D) Calcium induced release of cytochrome C (Cyt C) from mitochondria of cells indicated in (A, B). Cyt C was measured by western blot, with cytochrome oxidase I (COX1) serving as loading control. Blots shown are representative of three independent experiments.

(E, F) Calcium induced swelling of mitochondria from cell lines indicated in (A, B). Mitochondrial swelling was assessed by measuring changes in absorbance at 540nm. Error bars indicate standard deviation, *P < 0.001 versus vector by student's t-test.
See also Figure S2

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

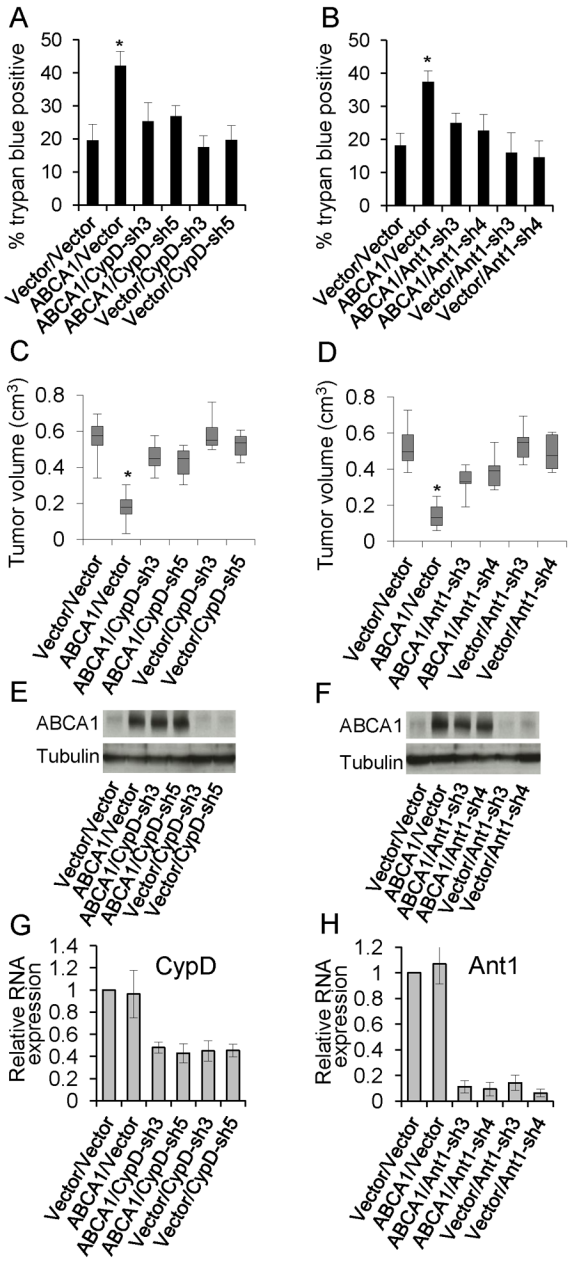

Figure 3. Suppression of CypD or Ant1 Restores Death Resistance of ABCA1-Reconstituted mp53/Ras Cells *in vitro* and Rescues Tumor Growth *in vivo*

(A, B) Cell death of the indicated derivatives of mp53/Ras cells: vector control (Vector/Vector), ABCA1-reconstituted (ABCA1/Vector), ABCA1-reconstituted plus Cyclophilin D knockdown (ABCA1/CypD-sh3 and -sh5) or Adenine Nucleotide Translocator 1 knockdown (ABCA1/Ant1-sh3 and -sh4), Cyclophilin D knockdown (Vector/CypD-sh3 or -sh5), and Adenine Nucleotide Translocator 1 knockdown (Vector/Ant1-sh3 or -sh4). Cell death was measured by trypan blue exclusion. Values represent the average of three independent experiments and error bars indicate standard deviation. $*P < 0.01$ versus all other cell lines, by student's t-test.

(C, D) Box plots indicating the volumes of tumors four weeks after subcutaneous implantation of the mp53/Ras cell derivatives indicated in (A, B). Each box shows the range

from the first to third quartile of the tumor volumes. The line in the box indicates the median tumor volume. The bars represent the largest and smallest tumors. *$P < 0.01$ versus all other cell lines, by student's t-test.
(E, F) Western Blots showing expression of ABCA1. Tubulin serves as a loading control.
(G, H) Expression of CypD, and Ant1 was measured by real time quantitative PCR. Error bars indicate standard deviation.
See also Figure S3

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

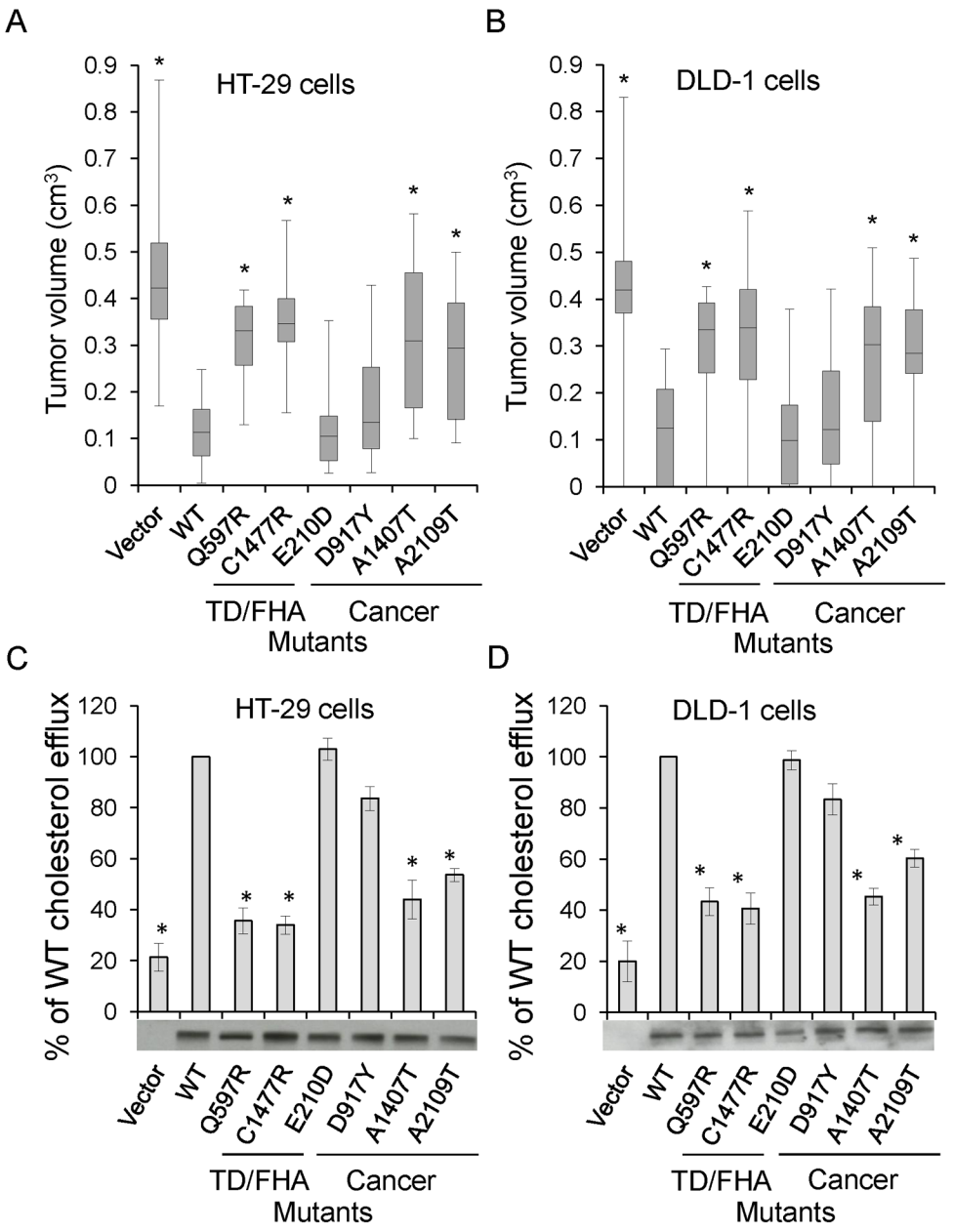

Figure 4. Loss of ABCA1 Efflux Function Correlates with Reduced Tumor Suppression

(A, B) Box plots indicating the volumes of tumors five weeks after subcutaneous implantation of the indicated derivatives of human colon cancer cell lines: (A) HT-29 and (B) DLD-1 expressing wild type human ABCA1 (hABCA1 WT) or the indicated ABCA1 mutants. Each box shows the range from the first to third quartile of the tumor volumes. The line in the box indicates the median tumor volume. The bars represent the largest and smallest tumors. *$P < 0.01$ versus WT hABCA1 expressing cell line, by student's t-test. (C, D) Cholesterol efflux capacities of hABCA1 mutants expressed in (A) HT-29 and (B) DLD-1 cells relative to hABCA1 wild type. Values indicate percentage of wild type efflux and represent the average of three independent experiments. Error bars indicate standard deviation. *$P < 0.01$ versus WT hABCA1 expressing cell line, by student's t-test. Images

below each graph are western blots showing representative protein expression of WT and mutant hABCA1 in cell lines used in (A, B, C, and D).
See also Figure S4

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript