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Deepa V. Dabir

Loyola Marymount University, deepa.dabir@lmu.edu

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Review

Clinical Implications of Augmenter of Liver Regeneration in Cancer: A Systematic Review

KEVIN H. NGUYEN, AUSTIN H. NGUYEN and DEEPA V. DABIR

Department of Biology, Loyola Marymount University, Los Angeles, CA, U.S.A.

Abstract. *Background/Aim: Hepatocellular carcinoma is a substantial healthcare burden with high prevalence and poor prognosis. As such, efforts are continually made to uncover molecules relevant in cancer biology, that are exploitable as targets for therapy. The mitochondrion is the powerhouse of the cell and exhibits altered functionality in the malignant state, including aberrant regulation of apoptosis and cellular respiration. Augmenter of liver regeneration (ALR) is a multifunctional mitochondrial protein that demonstrates anti-oxidative and anti-apoptotic properties and plays a key role in liver regeneration. Materials and Methods: The present study systematically reviews the available literature on the role of ALR in cancer. Results: Systematic search of PubMed resulted in 12 studies discussing ALR in multiple types of cancer. More specifically, ALR appears to be up-regulated in malignant cells and tissues. Furthermore, treatment of cells with exogenous ALR shows an anti-apoptotic effect while silencing or inhibiting ALR decreases cell and tumor survival. Conclusion: ALR clearly plays a role in cancer biology and demonstrates potential as a therapeutic target.*

Hepatocellular carcinoma (HCC) is a common oncologic condition with a poor prognosis that poses as a global burden on medical care (1). Between 2002 and 2012 the mortality rate of HCC rose by 34.8% in the United States and liver cancer, with HCC estimated as 70 to 90% of cases, was considered to be the second leading cause of cancer-related deaths among males on a global scale in 2012 (2, 3). Considering the metabolic aspect of several known risk

factors for HCC, such as obesity, diabetes, and non-alcoholic fatty liver disease, a common underlying feature of mitochondrial dysfunction is of growing interest in molecular oncology. The regulation of specific mitochondrial proteins is of particular interest, as these proteins demonstrate potential as therapeutic targets for HCC (4).

Augmenter of liver regeneration (ALR) is one particular protein of interest that localizes in the intermembrane space of the mitochondria of humans and has a prokaryotic homolog, Erv1, sharing 42% identical amino acid residues. ALR/Erv1 has been known to contribute to mitochondrial biogenesis and function as a sulfhydryl oxidase in mitochondrial disulfide relay systems (5). It functions to oxidize the import receptor Mia40, both of which conjunctively import small translocase inner membrane (Tim) proteins through an oxidative-folding pathway (6). In turn, cytochrome *c* (cyt *c*), which constitutes an important member of the electron transport chain, oxidizes ALR through locomotion of electrons *via* FAD, thus connecting this redox regulated import pathway to electron transport (5, 7). During specific unfavorable conditions, cells must reprogram to undergo apoptosis during which cyt *c* activates a series of activated-caspase pathways that lead to cell suicide (8). It is a trademark of cancer cells to down-regulate apoptotic mechanisms and alter mitochondrial respiratory function, resulting in uncontrolled growth and eventual metastasis of cancerous cells. ALR manifests anti-oxidative and anti-apoptotic properties and is known to support liver regeneration in cases of hepatic injury (9). Considering these properties, early investigations have suggested a role for ALR in cancer (particularly hepatocellular) pathogenesis, growth and progression. In this study, we systematically review the literature discussing ALR in cancer. Clinical implications including oncogenesis and targeted therapy are explored.

This article is freely accessible online.

Correspondence to: Dr. Deepa V. Dabir, 1 LMU Drive, Department of Biology, Loyola Marymount University, Los Angeles, CA 90045, USA. Tel: +1 3103384200, e-mail: ddabir@lmu.edu

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Materials and Methods

A systematic review was performed using the National Library of Medicine PubMed database. The search was performed using the following key terms: cancer and augmenter of liver regeneration, growth factor erv1-like, GFER, or hepatic regenerative stimulation

Table I. Summary of study details investigating ALR in cancer.

Study	Cancer type	N (total)	N (cancer)	Gender M:F	Mean age (range)	Tissue	Assays
Cao <i>et al.</i> , 2009	HCC	11	11	9:2	54.1 (37-66)	Frozen	qPCR, H2DCF-DA, flow cytometry
Dayoub <i>et al.</i> , 2011	HCC	64	53	-	-	Cell line HepG2, FFPE	IHC, migration/invasion assays, RT-PCR, WB
Denk <i>et al.</i> , 2010	HCC	-	-	-	-	Cell line HuH-7	WB, PCR, formazan formation cell viability assay
Gandhi <i>et al.</i> , 2015	HCC	-	-	-	-	FFPE, mice	IHC, WB, RT-PCR, transmission electron microscopy
Gatzidou <i>et al.</i> , 2015	CRC	42	23	11:12	68.8	Cell lines, frozen tissue	qPCR
Ilowski <i>et al.</i> , 2011	Various	-	-	-	-	Cell lines	WB, FACS
Polimeno <i>et al.</i> , 2009	Neuroblastoma	-	-	-	-	Cell line SH-SY5Y	Cell viability, flow cytometry, ICC, WB
Polimeno <i>et al.</i> , 2012	Glioma	-	-	-	-	Cell line T98G	WB, ICC, flow cytometry, electron microscopy
Shen <i>et al.</i> , 2013	T cell leukemia	-	-	-	-	Cell line Jurkat	Cell viability, flow cytometry, WB
Tang <i>et al.</i> , 2009	HCC	-	-	-	-	Cell line HepG2, mice	ICC, RT-PCR, mouse tumor model
Thasler <i>et al.</i> , 2005	HCC	71	9	5:4	66.2	FFPE, cell line HepG2	IHC, RT-PCR, WB
Yu <i>et al.</i> , 2010	HCC	Serum: 72 Tissue: 58	Serum: 20 Tissue: 6	-	-	Serum, FFPE	ELISA, RT-PCR, IHC

ALR: Augmenter of liver regeneration; CRC: colorectal cancer; FACS: fluorescence-activated cell sorting; FFPE: formalin-fixed paraffin embedded; HCC: hepatocellular carcinoma; ICC: immunocytochemistry; IHC: immunohistochemistry; PCR: polymerase chain reaction; RT-PCR: reverse-transcription polymerase chain reaction; WB: western blot.

substance. Articles were screened by titles and abstracts for possible relevance. Subsequently, full-text articles were retrieved for final inclusion decision. Inclusion criteria were: discussion of ALR in cancer, written in English, use of human subjects/samples. Conference abstracts and literature reviews were excluded from the present study.

Results

Systematic search of PubMed initially returned 41 articles, shown in Figure 1. Screening by titles and abstracts eliminated 28 articles. Full-text review eliminated one article that was an editorial and did not contain original data. Ultimately, 12 articles were included in the present review (10-21). Study characteristics are summarized in Table I.

Hepatocellular carcinoma. Results of studies investigating ALR in cancer are summarized in Table II. The majority of included studies observe ALR to be upregulated in malignant cells. In frozen and paraffin-embedded HCC tissue, ALR expression is consistently altered when compared to normal para-tumoral tissue and/or healthy controls (10, 11, 13, 20, 21). The majority of studies suggest ALR mRNA and protein levels to be higher in HCC tissue relative to normal tissue. In HCC tissue, ALR typically demonstrates cytoplasmic localization with a granular quality on immunostaining (20). Dayoub and colleagues, however, observe the short ALR isoform of 15 kDa to be expressed at lower levels in HCC overall, compared to normal liver tissue (11). Furthermore,

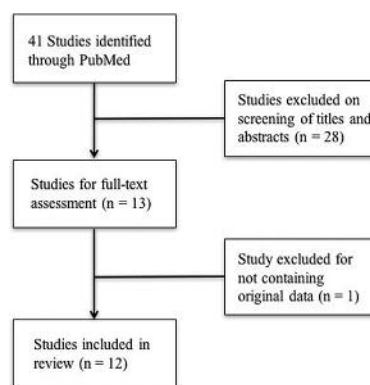


Figure 1. Systematic review of PubMed for articles discussing the clinical value of ALR in cancer. Initial search returned 41 articles. Screening by titles, abstracts, and full texts resulted in 12 articles remaining for final inclusion in this review.

this study found ALR expression to be inversely correlated with tumor grade and histologic angioinvasion. No other studies specified the isoform of ALR investigated. Additionally, one study demonstrated serum ALR levels to be unchanged in patients with HCC (21).

The bulk of studies investigated the role of ALR in cancer biology using HCC cell lines. *In vitro*, ALR is consistently associated with a protective effect on cancer cell survival and

Table II. Summary of clinical implications of ALR in cancer.

Study	Cancer	Findings
Cao <i>et al.</i> , 2009	HCC	<ul style="list-style-type: none"> • ALR is up-regulated by greater than twofold in 81.9% of HCC specimens, compared to adjacent noncancerous tissue • Overexpression of ALR in HCC cell lines was associated with increased survival after irradiation, attenuates post-irradiation mitochondrial dysfunction, and inhibits mitochondria-dependent apoptosis • ALR silencing inhibited growth and survival of HCC lines, but had minimal effect on normal hepatocyte cells
Dayoub <i>et al.</i> , 2011	HCC	<ul style="list-style-type: none"> • The short ALR isoform of 15 kDa is expressed less in HCC cells, compared to normal liver tissue • Reexpression of the 15 kDa ALR isoform in HCC cells reduces migration and invasiveness and attenuates tumorigenicity • ALR expression in HCC tissue inversely correlates with tumor grade and rate of histological angiogenesis
Denk <i>et al.</i> , 2010	HCC	<ul style="list-style-type: none"> • Transfection of HCC cells with ALR does not decrease apoptosis after treatment with glycochenodeoxycholic acid
Gandhi <i>et al.</i> , 2015	HCC	<ul style="list-style-type: none"> • Liver-specific ALR knock-out mice demonstrated decreased mitochondrial respiratory function, defective mitochondrial fatty acid transport and ATP synthesis • They also demonstrated increased oxidative stress, steatosis, hepatocyte apoptosis, mitochondrial structural abnormalities • HCC developed in nearly 60% of liver-specific ALR knock-out mice by 1 year • ALR levels were lower in patients with advanced alcoholic liver disease and nonalcoholic steatohepatitis compared to controls
Gatzidou <i>et al.</i> , 2015	CRC	<ul style="list-style-type: none"> • ALR was expressed at higher levels in CRC cell lines compared to controls • ALR expression was significantly increased in well/moderately differentiated tumors compared to poorly differentiated tumors • ALR tended to be higher in CRC tissue compared to matched non-neoplastic tissue
Ilowski <i>et al.</i> , 2011	Various	<ul style="list-style-type: none"> • HCC cells cultured in rhALR significantly decreased apoptosis induced by ethanol, TRAIL, anti-Apo, TGF-β, and actinomycin D • rhALR treatment did not reduce apoptosis in bronchial, colonic, gastric, and pancreatic cell lines
Polimeno <i>et al.</i> , 2009	Neuroblastoma	<ul style="list-style-type: none"> • Neuroblastoma cells cultured with ALR exhibited improved survival in H₂O₂-induced apoptosis
Polimeno <i>et al.</i> , 2012	Glioma	<ul style="list-style-type: none"> • Glioma cells treated with rhALR dose-dependently decreased ROS levels in H₂O₂-treated cells • Silencing of ALR-encoding mRNA decreased clusterin and bcl-2 levels and increased apoptosis and apoptotic markers caspase-9, activated caspase-3, ROS, and mitochondrial degeneration
Shen <i>et al.</i> , 2013	T cell leukemia	<ul style="list-style-type: none"> • Leukemic T cells show upregulation of ALR compared to normal T lymphocyte controls • Culture of leukemic T cells with ALR reduces sensitivity to vincristine treatment but does not effect proliferation
Tang <i>et al.</i> , 2009	HCC	<ul style="list-style-type: none"> • Silencing of ALR inhibited growth of HCC cells <i>in vitro</i> • Silencing of ALR and treatment of anti-ALR monoclonal antibodies both inhibited xenografted HCC tumors in nude mice
Thasler <i>et al.</i> , 2005	HCC	<ul style="list-style-type: none"> • HCC tissue demonstrated intense cytoplasmic granular immunostaining for ALR • ALR mRNA expression in HCC tissue was significantly higher compared with normal liver tissue samples
Yu <i>et al.</i> , 2010	HCC	<ul style="list-style-type: none"> • Serum ALR levels did not differ among patients with HCC, chronic hepatitis B, and healthy controls • ALR mRNA and protein levels were significantly higher in HCC liver tissues compared to healthy controls

ALR: Augmenter of liver regeneration; CRC: colorectal cancer; HCC: hepatocellular carcinoma; rhALR: recombinant human ALR; ROS: reactive oxygen species; TGF- β : transforming growth factor beta; TRAIL: tumor necrosis factor related apoptosis-inducing ligand.

apoptosis. Cao *et al.* observed overexpression of ALR in HCC cells to have a protective effect against radiation-induced mitochondrial dysfunction and subsequent apoptosis (10). Additionally, treatment of HCC cells with ALR has a protective effect against apoptosis induced by ethanol, TRAIL, anti-Apo, TGF- β , and actinomycin D (15).

Silencing of ALR consistently demonstrated an anti-tumoral effect in HCC. Upon ALR silencing, overall growth and survival of HCC cells was inhibited, while a minimal

effect was noted in normal hepatocyte cells (10, 19). In a murine HCC xenograft model, silencing of ALR and treatment with anti-ALR monoclonal antibodies both similarly inhibited tumor growth (19). Only one study reported that lowered ALR expression has been linked to HCC oncogenesis. In this study, mice with a liver-specific ALR knock-out were observed to have increased hepatosteatosis, hepatocyte apoptosis, and elevated HCC incidence of 60% at one year (13).

Other cancers. Five studies discussed cancers other than HCC (14, 15, 16, 17, 18). Gatzidou and colleagues observed elevated ALR levels in colorectal cancer (CRC) tissue and cell lines when compared to matched non-neoplastic tissue or controls, respectively (14). Similar to HCC, ALR expression in malignant tissue was inversely correlated with tumor grade. Namely, ALR was elevated in well to moderately differentiated tumors when compared to poorly differentiated ones. In another study, *in vitro* treatment with ALR does not appear to reduce apoptosis in bronchial, colonic, gastric, and pancreatic malignant cell lines (15). In T cell leukemia, cultured cells were observed to upregulate ALR compared to normal primary T lymphocytes (18). Furthermore, treatment with ALR reduces leukemic T cell sensitivity to vincristine.

ALR is of interest in neuronal tissue as well. Both neuroblastoma and glioma cells cultured with ALR exhibit a dose-dependent improvement in survival in H₂O₂-induced apoptosis (16, 17). Furthermore, silencing of ALR in glioma cells induces a pro-apoptotic state with increased reactive oxygen species generation and mitochondrial degeneration.

Discussion

Expression of ALR as assessed by varied qualitative and quantitative assays is clearly altered in malignancy. In light of the multifunctional nature of mitochondria, ALR likely plays a central role in cancer biology, in apoptotic regulation and cell respiration. In HCC tissues, ALR was consistently increased compared to healthy controls with the exception of one study which specifically investigated the 15 kDa isoform. *Via* alternative splicing, ALR may be found as a 21/23-kDa isoform predominantly in the mitochondrial inter membrane space or as a 15-kDa isoform at extracellular, nuclear, and cytoplasmic localizations (11). This likely accounts for the inconsistencies in ALR levels found across the included studies. In HCC, the malignant state may be associated with an altered expression profile of ALR that is preferential for the larger isoform. This would also explain the oncogenic effect of ALR knock-out in mice (13). If the 15-kDa isoform is associated with healthy hepatocytes while the longer isoform is upregulated in malignancy, a pan-ALR knock-out may be oncogenic. Further investigation dissecting the specific functions of the ALR isoforms in healthy cell and cancer biology is necessary.

ALR demonstrates potential as a therapeutic target in cancer treatment. Sufficient *in vitro* evidence suggests ALR silencing to be anti-tumoral. Silencing and/or inhibiting ALR in an HCC xenograft nude mouse model showed promising anti-tumor effect (19). However, considering oncogenic effect and increased hepatosteatosis in the murine liver-specific knock-out of ALR (13), further elucidation of the roles of the ALR isoforms must be performed. Perhaps specific targeting of the 21/23-kDa isoform may minimize adverse outcome.

Overall, the currently available literature clearly suggests an oncogenic role of ALR in cancer biology. However, the complete function of ALR in cancer is not fully understood. Further investigations need to be performed on ALR function in tumor progression and as a targeted therapy. This early evidence, however, is promising for clinical use of this molecule.

Conflicts of Interest

None to declare.

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