Cancer Letters 353 (2014) 258–263

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Original articles

Inhibition of fatty acid synthase induces pro-survival Akt and ERK signaling in K-Ras-driven cancer cells

Paige Yellen [1], David A. Foster *

Department of Biological Sciences, Hunter College, City University of New York, New York, NY, USA

ARTICLE INFO

Article history:
Received 14 May 2014
Received in revised form 15 July 2014
Accepted 16 July 2014

Keywords:
Fatty acid synthase
K-Ras
Akt
ERK

ABSTRACT

Cancer cells with constitutive phosphatidylinositol 3-kinase (PI3K)/Akt pathway activation have been associated with overexpression of the lipogenic enzyme fatty acid synthase (FAS) as a means to provide lipids necessary for cell growth. In contrast, K-Ras-driven cancer cells suppress utilization of *de novo* synthesized fatty acids and rely on exogenously supplied fatty acids for cell growth and membrane phospholipid biosynthesis. Consistent with a differential need for *de novo* fatty acid synthesis, cancer cells with activated PI3K signaling were sensitive to suppression of FAS; whereas mutant K-Ras-driven cancer cells continued to proliferate with suppressed FAS. Surprisingly, in response to FAS suppression, we observed robust increases in both Akt and ERK phosphorylation. Akt phosphorylation was dependent on the insulin-like growth factor-1 receptor (IGF-1R)/PI3K pathway and mTOR complex 2. Intriguingly, K-Ras-mediated ERK activation was dependent on N-Ras. Pharmacological inhibition of PI3K and MEK in K-Ras-driven cancer cells resulted in increased sensitivity to FAS inhibition. These data reveal a surprising sensitivity of K-Ras-driven cancer cells to FAS suppression when stimulation of Akt and ERK was prevented. As K-Ras-driven cancers are notoriously difficult to treat, these findings have therapeutic implications.

Introduction

Several studies have shown that endogenous lipid production is necessary for growth and survival in cancer cells of various tissue types and mutation signature [1–6]. A subset of research has focused on cancer cells with dysregulation of the phosphatidylinositol-3-kinase (PI3K)[2]/Akt pathway [7–9]. Among the many targets of PI3K signals, Akt induces lipogenic enzymes, including fatty acid synthase (FAS) [10], which catalyzes the terminal step in *de novo* lipogenesis, the anabolic conversion of glucose into fatty acids. Increased glucose uptake by cancer cells with constitutive PI3K/Akt signaling has been associated with high levels of FAS expression and increased fatty acid synthesis [11–13], thereby satisfying the demand for new membrane composition in rapidly proliferating cells. Constitutive Akt activation can be the result of a gain-of-function mutation in the PI3K gene (PIK3CA) [14] or more commonly, loss of PI3K antagonist, PTEN (phosphatase and tensin homologue deleted on chromosome 10). Loss of PTEN sensitizes cells to FAS inhibition [15,16] while induction of PTEN abrogates the effect [7,17]. The inference is that cancer cells with intact PTEN and corresponding low Akt activation and FAS expression are unaffected by FAS inhibition.

Despite intact PTEN, K-Ras-driven cancer cells can activate the PI3K/Akt pathway – making it difficult to target cancer cells harboring K-Ras mutations [18,19]. In addition to being able to activate the PI3K/Akt pathway [20,21], oncogenic K-Ras also activates the Raf/MEK/ERK pathway [22]. PI3K/Akt activation is also regulated by growth factors through a canonical insulin-like growth factor-1 receptor (IGF-1R)/PI3K/Akt pathway [23,24]. Whether cancer cells with oncogenic K-Ras are linked to the PI3K/Akt pathway directly (predictive of growth-factor independence) or indirectly (growth-factor dependent), the RAS/Raf/MEK/ERK and PI3K/Akt pathways are compensatory [25,26]. Thus, single agents targeting either pathway are not efficacious. Instead, combined inhibition of components in both pathways is necessary to compromise cancer cells with mutant K-Ras [27].

In this study, we investigated the effect of FAS inhibition on proliferation and viability of K-Ras mutant cancer cells. We used pharmacological and genetic means to inhibit FAS in human cancer cell lines harboring K-Ras mutations. We found a surprising tumorigenic advantage in that Fas inhibition led to Akt and ERK activation. Because tumors adapt to a nutrient-depleted

Abbreviations: FAS, fatty acid synthase; IGF-1R, insulin-like growth factor-1 receptor; mTORC2, mammalian/mechanistic target of rapamycin complex 2; PI3K, phosphatidylinositol 3-kinase.

* Corresponding author. Hunter College, City University of New York, 695 Park Avenue, New York, NY 10065, USA. Tel.: +1 212 772 4075; fax: +1 212 772 5227.
E-mail address: foster@genectr.hunter.cuny.edu (D.A. Foster).
[1] Present address: Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

http://dx.doi.org/10.1016/j.canlet.2014.07.027
0304-3835/

microenvironment during tumorigenesis, these findings identify survival signals that may need to be compromised for therapeutic intervention.

Materials and methods

Cells, cell culture conditions and cell viability

The human cancer cell lines PANC-1, Mia PaCa-2, HCT116, MDA-MB-468 and PC3 cells were obtained from the American Tissue Type Culture Collection and cultured in Dulbecco's Modified Eagle Medium (Sigma) supplemented with 10% Fetal Bovine Serum (Sigma). Cell viability was determined as ratio of non-adherent cells to adherent cells after treatment using a Coulter counter.

Antibodies and reagents

The following antibodies against: PARP, PTEN, Akt, P-Akt^{S473}, P-ERK, ERK, P-P70 S6 kinase, mTOR, Raptor, Rictor, and IGF-1R were obtained from Cell Signaling; α-actin was from Sigma. The antibody for FAS was obtained from BD BioSciences. Negative control scrambled siRNA and siRNA targeted against mTOR, Raptor and Rictor were obtained from Dharmacon. siRNAs targeted against FAS were obtained from Santa Cruz Biotechnology. Cerulenin, LY294002 and PD0325901 were purchased from Sigma. Lipofectamine RNAiMax (Invitrogen) was used for transient transfections.

Cell proliferation

Cells were plated at 50% confluence and treated the next day. Cells were trypsinized at 24 and 48 hours and counted using a Coulter counter.

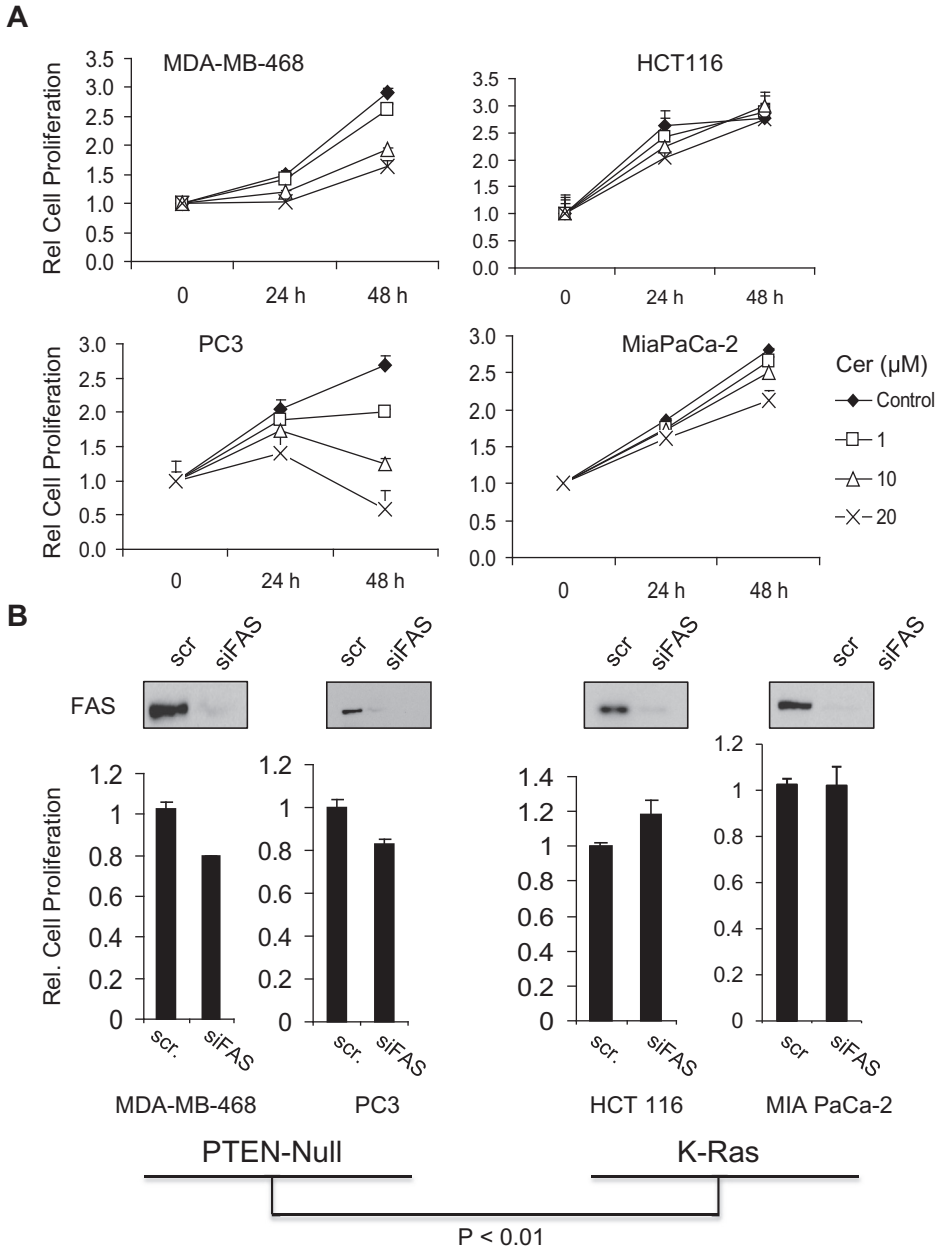

Fig. 1. K-Ras mutant cancer cells proliferate independently of fatty acid synthase inhibition. (A) PTEN null cells lines, MDA-MB-468 and PC3 and K-Ras mutant cell lines, HCT116 and MIA PaCa-2 cells were plated at 50% confluence and treated with cerulenin (Cer) at indicated doses. After 24 and 48 hour time points, cells were trypsinized and counted by Coulter counter. Data represent mean ± SEM from three independent experiments with each sample counted two times. (B) Cells were transiently transfected with 100 nM siRNA against FAS (siFAS) or with negative control scrambled siRNA (scr). After 48 hours, cells were harvested and lysates were immunoblotted for FAS protein and counted by Coulter counter. Data represent mean ± SEM from three independent experiments with each sample counted two times. P-value represents combined data sets for the PTEN-null cell lines (MDA-MB-468 and PC3) and K-Ras mutant cell lines (HCT116 and MIA PaCa-2). The values for the ratios of cell number in cells treated with siFAS divided by cell number of controls in the PTEN-null and mutant K-Ras cell lines.