Biochimica et Biophysica Acta xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Metabolic synthetic lethality in cancer therapy☆

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ARTICLE INFO

Article history:
Received 29 September 2016
Received in revised form 23 November 2016
Accepted 5 December 2016
Available online xxxx

ABSTRACT

Our understanding of cancer has recently seen a major paradigm shift resulting in it being viewed as a metabolic disorder, and altered cellular metabolism being recognised as a hallmark of cancer. This concept was spurred by the findings that the oncogenic mutations driving tumorigenesis induce a reprogramming of cancer cell metabolism that is required for unrestrained growth and proliferation. The recent discovery that mutations in key mitochondrial enzymes play a causal role in tumorigenesis suggested that dysregulation of metabolism could also be a driver of tumorigenesis. These mutations induce profound adaptive metabolic alterations that are a prerequisite for the survival of the mutated cells. Because these metabolic events are specific to cancer cells, they offer an opportunity to develop new therapies that specifically target tumour cells without affecting healthy tissue. Here, we will describe recent developments in metabolism-based cancer therapy, in particular focusing on the concept of metabolic synthetic lethality. This article is part of a Special Issue entitled Mitochondria in Cancer, edited by Giuseppe Gasparre, Rodrigue Rossignol and Pierre Sonveaux.

1. Introduction

Cancer is thought to arise through sequential genetic changes that ultimately convert a normal cell to a fully transformed one. However, research over the past decade revealed that the process of transformation is accompanied by profound metabolic changes, adding deregulation of cellular energetics to the list of emerging hallmarks of cancer [1]. The discovery that tumour cells undergo metabolic re-programming was first reported by Otto Warburg almost a century ago [2] but the connection between cancer and metabolism was temporarily eclipsed by the discovery of the role of oncogenes and tumour suppressors in cancer. However, recent findings demonstrating a link between the oncogenic mutations that drive tumorigenesis and the mechanistic basis of cancer metabolism have led to renewed interest in the field [3]. It is now established that oncogenic pathways such as those supported by RAS or MYC promote glycolysis [4], while tumour suppressors like p53 inhibit it [5]. Beside these major pathways, a number of metabolic enzymes has been found to be mutated in cancer [6]. These findings suggest that dysregulation of metabolism is not only a consequence, but also a cause of tumorigenesis [7]. If on one hand the metabolic reprogramming is required to support growth and proliferation of cancer cells, on the other hand it exposes their Achilles' heel. Indeed, since these metabolic events are specific to cancer cells, they offer an opportunity to develop new selective therapies that would target tumour cells without affecting healthy tissue. In this review we will describe recent developments in metabolism-based cancer therapy, focusing on the concept of metabolic synthetic lethality.

2. Targeting cancer metabolism

2.1. Anti-metabolic therapy

The idea of targeting cancer metabolism traces back to the original work of Sydney Farber, who, after some failed attempts, for the first time successfully used inhibitors of folate synthesis to kill leukaemia cells [8]. Despite having some toxicity in normal proliferative tissues such as the intestinal epithelium and bone marrow, antifolates are still effectively used in combined therapies to target increased nucleotide and DNA synthesis in tumour cells [9]. Since then, the so-called antimetabolites, *i.e.* anticancer drugs that affect nucleotide biosynthesis, have been amongst the most successful drugs for cancer therapy [10]. However, the metabolic reprogramming of cancer goes beyond altered nucleotide biosynthesis.

The first of the metabolic features to be identified in tumour cells was an increase in glucose uptake and glycolysis leading to an increase in lactate production [11]. Although, this feature is specific to cancer cells it still involves many of the same glycolytic enzymes used by normal cells, making it a difficult cancer-specific target. Nonetheless, some enzymes are preferentially used by cancer cells and their targeting has yielded positive results. These include glucose transporters (such as GLUT1), hexokinases (such as HK2), phosphofructokinases (such as PFK1), pyruvate kinase (PKM2) and intracellular lactate transporters (monocarboxylate transporters MCT1 and MCT4) [6,9].

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http://dx.doi.org/10.1016/j.bbabio.2016.12.003
0005-2728/

Glycogen is a multi-branched polysaccharide of glucose that is used by the cell as energy storage. Its metabolism was showed to be up-regulated in many tumour types and to play a role in metabolic reprogramming of tumour cells under stress conditions such as glucose deprivation or hypoxia [12]. Depletion of glycogen phosphorylase, an enzyme that catalyzes glycogen degradation, has been shown to impair tumorigenesis *in vivo*. Its expression is increased *in vivo* following treatment with bevacizumab, an angiogenesis inhibitor [13], suggesting that targeting this pathway in hypoxic tumours might be effective.

In addition to the widely studied dysregulated glucose metabolism, it has become apparent that many other metabolites are also used by cancer cells to fuel tumorigenesis including a wide array of amino acids such as glutamine, serine, glycine, proline, and arginine [14]. High levels of *de novo* serine biosynthesis have been observed in some cancer cells and suppression of phosphoglycerate dehydrogenase (PHGDH) activity, an enzyme involved in serine biosynthesis, preferentially inhibits the proliferation of these serine-dependent cells [14]. Of note, novel PHGDH inhibitors have been recently developed [15,16] and they exhibit excellent antiproliferative properties in PHGDH-amplified cancer cells.

In many cancers, glutamine is used as a major source of carbon and nitrogen, and approaches that target glutamine metabolism have been investigated as a potential way to reduce cancer cell survival [17]. For example, inhibition of the glutaminase 2 (GLS2) with small-molecule inhibitor such as alkyl benzoquinone AV-1, suppresses oncogenic transformation of cancer cell lines without affecting the growth of normal cells [18]. Furthermore, a combination of glutamine-analogue acivicin and GLS2 inhibitor synergistically reduces the proliferation and invasion of some cancer cell types [19].

Glutamine and proline metabolism are interlinked and recent studies show that the regulation of proline metabolism plays a role in carcinogenesis [20,21]. Proline biosynthesis from glutamine is increased by MYC [22] through up-regulation of the expression of the enzymes involved in its biosynthesis pathway [23]. Blockade of proline biosynthesis decreases tumour cell growth and energy production, and recent evidence shows that proline oxidase, an enzyme catalyzing the first step of proline degradation, plays an important role in tumorigenesis and tumour growth by maintaining pyridine nucleotide levels [23,24].

Asparagine is another key metabolite for cancer cells [6]. Consistently, another example of amino acid-based anti-metabolic therapy for cancer is asparaginase, an enzyme that converts asparagine to aspartic acid. Asparaginase is used in the treatment of acute lymphocytic leukaemia (ALL), where cancer cells are characterised by a poor ability to synthesize asparagine *de novo* due to suppression of asparagine synthetase (ASNS). The systemic administration of asparaginase eliminates the exogenous supply of asparagine and selectively affects ALL cells [25]. Interestingly, asparagine deprivation and the silencing of ASNS have been identified as key liabilities for sarcoma in a functional genomic screening [26]. Similarly, arginine-depleting strategies are used for cancer cells that cannot synthesise arginine due to suppression of the urea cycle enzyme argininosuccinate synthetase 1 (ASS1). Among the commonest treatment strategies for arginine depletion are pegylated arginine deiminase (ADI-PEG20), an FDA approved drug in advanced clinical trials for hepatocellular carcinoma, melanoma and prostate cancer and mesothelioma cells, and recombinant human arginase, a drug in early phase I clinical trials for melanoma, renal cell carcinoma, prostate cancer and hepatocellular carcinoma [27].

Lipid metabolism has also been shown to play a central role in tumorigenesis [28] and studies have demonstrated the efficacy of inhibition of lipogenic enzymes such as fatty acid synthase (FASN), ATP citrate lyase (ACLY) and Acetyl-CoA carboxylase (ACC) using Orlistat, SB-204990 and TOFA, respectively, as anticancer therapies in various preclinical models of carcinogenesis [29,30].

Targeting metabolic pathways seems a promising cancer-specific therapy. However, this strategy has not fully translated into compelling clinical results [31]. It is possible that, given the ubiquitous nature of metabolic reactions and the flexibility of the metabolic network, metabolism is more difficult to target than originally anticipated. Hence, alternative strategies have been recently proposed. This is where the concept of synthetic lethality has gained traction.

2.2. Metabolic synthetic lethality, a novel approach for cancer therapy

First reported in the early 20th century by the American geneticist Calvin Bridges, synthetic lethality (SL) describes a genetic interaction whereby the combination of mutations in two or more separate genes results in a lethal phenotype, whereas mutation of each gene individually does not affect cellular or organismal viability [32–34]. SL interactions are more commonly associated with loss of function alleles but can also apply to gain of function alleles; the process is then known as synthetic dosage lethality (SDL). SDL denotes a genetic interaction whereby silencing of a gene combined with the overexpression of another gene is lethal to the cell. As cancer cells often overexpress specific tumour-driving oncogenes that are difficult to target directly, silencing their SDL partners may result in specific elimination of these cells. By exploiting the intrinsic differences between cancer and healthy cells, SL-based therapeutic approaches can specifically target tumour cells in a way that is often not achievable using conventional therapeutic drugs. Recent technological developments in genome-wide profiling made possible the systematic screening for SL interactions in human cells using small molecule inhibitors or high-throughput RNAi-based screens. These screens have been successfully used to identify SL pathways associated with known tumour suppressors and oncogenes [35]. One classic example is the inhibition of poly (ADP-ribose) polymerase in cancer cells that harbour breast cancer genes *BRCA1* and *BRCA2* loss-of-function mutations resulting in specific lethality to these cells [36]. The *BCR-ABL* fusion oncogene is a major driver of tumorigenesis in chronic myelogenous leukaemia. Addiction of the tumour cells to the constitutively active BCR-ABL protein kinase results in sensitization to imatinib [37] and a recent screen identified a SL interaction between STAT3 and BCR-ABL [38]. The MYC oncogene contributes to the formation of a large number of cancers. A recent study aimed at identifying SL partners of oncogenic MYC has revealed that the most over-represented functional categories among *MYC* SL genes are DNA-repair and cell cycle [39]. In that context, bromodomain-containing proteins Cat Eye Syndrome Chromosome Region, Candidate 2 (CECR2), BRCA1-associated RING domain protein 1 (BARD1) and ATPase Family, AAA Domain Containing 2 (ATAD2) as well as cyclin-dependent kinase 12 (CDK12) recently emerged as synthetic lethal interactors of MYC [40–42]. In addition, a SL RNAi screen using MYC overexpression also highlighted synthetic dosage lethal MYC partners, such as the ubiquitin ligase FBXW7 [40].

Recently, the concept of SL has been applied to cellular metabolism. For example, AMPK-related kinase 5 (ARK5) was shown to be SL with oncogenic MYC *via* activation of the mammalian target of rapamycin (mTOR) pathway [43]. In oncogenic MYC-expressing cells, inhibition of ARK5 results in a drop in ATP levels and leads to subsequent induction of pro-apoptotic responses. In addition, depletion of ARK5 in MYC-driven mouse models of hepatocellular carcinoma extends survival and demonstrates the therapeutic value of this synthetic lethal interaction. As discussed above, a recurrent metabolic feature of cancer cells is an increase in glycolysis. Activation of this pathway generates high levels of lactate from pyruvate through lactate dehydrogenase A (LDH-A). This reaction reduces nicotinamide adenine dinucleotide (NAD^+) into NADH, which is used to transport electrons to the electron transport chain (ETC). The Combination of LDH-A inhibition with small inhibitor FX11 and FK866, a NAD^+ synthesis inhibitor, reduces the NAD^+ cellular pool in lymphoma resulting in tumour regression [44].

Because of the intricacy of metabolic networks and the complexity with which these are reprogrammed in cancer cells, *in silico* modelling approaches to investigate SL interactions have also been proposed [45]. For instance, in a recent study, a genome-wide network model of

Please cite this article as: V. Zecchini, C. Frezza, Metabolic synthetic lethality in cancer therapy, Biochim. Biophys. Acta (2016), http://dx.doi.org/10.1016/j.bbabio.2016.12.003