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Mini-review

Adaptations of energy metabolism during cerebellar neurogenesis are co-opted in medulloblastoma

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ABSTRACT

Recent studies show that metabolic patterns typical of cancer cells, including aerobic glycolysis and increased lipogenesis, are not unique to malignancy, but rather originate in physiologic development. In the postnatal brain, where sufficient oxygen for energy metabolism is scrupulously maintained, neural progenitors nevertheless metabolize glucose to lactate and prioritize lipid synthesis over fatty acid oxidation. Medulloblastoma, a cancer of neural progenitors that is the most common malignant brain tumor in children, recapitulates the metabolic phenotype of brain progenitor cells. During the physiologic proliferation of neural progenitors, metabolic enzymes generally associated with malignancy, including Hexokinase 2 (Hk2) and Pyruvate kinase M2 (PkM2) configure energy metabolism to support growth. In these non-malignant cells, expression of Hk2 and PkM2 is driven by transcriptional regulators that are typically identified as oncogenes, including N-myc. Importantly, N-myc continues to drive Hk2 and PkM2 in medulloblastoma. Similarly E2F transcription factors and PPARγ function in both progenitors and medulloblastoma to optimize energy metabolism to support proliferation. These findings show that the "metabolic transformation" that is a hallmark of cancer is not specifically limited to cancer. Rather, metabolic transformation represents a co-opting of developmental programs integral to physiologic growth. Despite their physiologic origins, the molecular mechanisms that mediate metabolic transformation may nevertheless present ideal targets for novel anti-tumor therapy.

1. Introduction

Many cancers demonstrate metabolic transformation, configuring cellular metabolism to support malignant growth [1]. Metabolic patterns commonly observed in cancer include increased lipogenesis [2–4] and aerobic glycolysis, the metabolism of glucose to lactate, despite the availability of oxygen for oxidative phosphorylation [5,6]. Up-regulation of aerobic glycolysis in cancer is known as the Warburg effect and is understood to be a malignant adaptation that allows continued proliferation in diverse microenvironments. The importance of metabolic transformation to cancer pathogenesis, however, raises important questions: Are these metabolic programs unique to cancer cells? If not, what might be their physiologic origins? Recent studies of postnatal neurogenesis have identified a physiological role in neural development for metabolic patterns typically associated with cancer, including both increased lipogenesis [7,8] and aerobic glycolysis [8,9]. In contrast to the production of aberrant onco-metabolites such as 2-hydroxyglutarate, which requires IDH mutation [10–12], the lipogenic and glycolytic phenotypes frequently observed in cancer originate in the normal metabolic repertoire of neural progenitor cells. Neurogenesis, like cancer, involves rapid proliferation and these studies show that metabolic pathways in neural progenitors, as in cancer cells, are optimized for cell division. The pattern of increased lipogenesis and aerobic glycolysis manifest neural progenitors, is maintained in the progenitor-derived brain tumor medulloblastoma; in this cancer, developmentally-regulated metabolism is co-opted to support malignant growth. These studies provide a developmental perspective on the origins of cancer cell metabolism.

Medulloblastoma is the most common malignant brain tumor in children, and presents an ideal opportunity to examine cancer arising as a disruption of developmentally-regulated growth [13]. Medulloblastomas originate from the cerebellum, which is the

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most prominent site of neural progenitor proliferation in early post-natal life. Several lines of evidence link cerebellar neural progenitor proliferation to medulloblastoma pathogenesis. In the first year of life in humans, or the first 15 days of life in mice, cerebellar granule neuron progenitors (CGNPs) proliferate in a germinal matrix along the outside of the cerebellum called the external granule cell layer (EGL). This period of rapid proliferation is triggered by activation of the Sonic Hedgehog (Shh) signaling pathway [14]. While proliferation of cerebellar progenitors is virtually shut down once cerebellar development is complete, mutations in humans that aberrantly activate the Shh pathway predispose individuals to medulloblastoma formation; importantly, this process is recapitulated in transgenic mice [15–17]. Thus mice with conditional deletion of Patched (Ptc) or constitutively active alleles of Smoothened (Smo) allow the process of medulloblastoma tumorigenesis process to be examined prospectively, from CGNP proliferation forward to cancer [17,18].

Along with induction of proliferation in the postnatal cerebellum, Shh signaling induces characteristic metabolic patterns in CGNPs, including decreased fatty acid oxidation [7], increased lipogenesis [7] and aerobic glycolysis [8,9]. Despite the normoxic environment of the postnatal brain, Shh drives a shift in energy production away from oxidative reactions. This developmentally-programmed metabolic configuration of CGNPs persists in primary medulloblastoma in transgenic mice [7–9]. Studies of human patients, moreover, show that the glycolytic phenotype of the model is shared by the actual disease; medulloblastomas are readily detected by clinical ^{18}FDG-PET studies [19,20] and glucose uptake correlates inversely with patient survival [19]. Thus understanding the cellular and molecular mechanisms of metabolic configuration of neural progenitors places the metabolic patterns of medulloblastoma into a developmental context and may provide key insight in tumor pathogenesis.

Bhatia et al demonstrated that Shh induced a metabolic switch from lipid consumption to lipid production [7]. After observing abundant lipid deposition in Shh-driven medulloblastomas in transgenic mice, the investigators examined whether fatty acid metabolism was altered by Shh in CGNPs, the normal cells from which these tumors originate. Bhatia et al found that CGNPs explanted into media containing Shh up-regulated key lipid synthesis enzymes, including Fatty Acid Synthase (FASN) and Acetyl-CoA Carboxylase (Acc1). Shh also caused down-regulation of enzymes required for lipid catabolism, including Acyl-CoA Oxidase 1 (Acox1) and Medium Chain Acyl-CoA Dehydrogenase (MCAD) [7]. These transcriptional changes seemed to be coupled with proliferation as they depended on the activity of the Rb-E2F axis; E2F1 knockdown blocked the induction of FASN and the suppression MCAD in Shh-treated CGNPs. Direct measurement of palmitate oxidation demonstrated that these transcriptional changes potently altered lipid metabolism; Shh reduced palmitate oxidation, which could be restored by the subsequent addition of E2F1 shRNA. Thus, as depicted in Fig. 1, Shh shifted the lipid metabolism of CGNPs from catabolic to synthetic, and this shift was mediated by E2F1 and negatively regulated by Rb [7].

Importantly, this metabolic switch was maintained in medulloblastoma, where it promoted tumor growth [7]. Treatment of medulloblastoma-bearing mice with inhibitors of either FASN or the CDK-Rb-E2F signaling pathway slowed medulloblastoma progression and prolonged mouse survival. Bhatia et al used the transgenic ND2:SmoA1 mouse line to generate animals with primary medulloblastoma. After tumor formation, these mice were injected daily for 2 weeks with either the CDK inhibitor olomoucine or with the FASN inhibitor C75. Both agents significantly extended animal survival by slowing tumor growth. Within treated tumors, C75 reduced lipid synthesis as expected. Similarly, olomoucine reduced intratumoral abundance of FASN [7]. Thus, direct inhibition of fatty acid synthesis exerted a significant anti-tumor effect and this effect was duplicated through CDK inhibition. Together these data show that lipogenic metabolism is both essential to tumor growth and derives from developmental physiology [7].

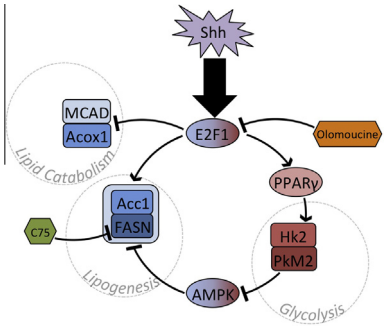

Fig. 1. Shh signaling regulates lipid metabolism and glycolysis in an integrated manner. Regulatory genes and effector proteins are denoted by ovals and rounded rectangles, respectively, whereas inhibitors are in hexagons. Entities involved in lipid or glucose metabolism are in blue or red. AMPK may switch-off lipogenesis when aerobic glycolysis is blocked and energy scarcity results. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The same group, in a follow up investigation, demonstrated that the metabolic regulator, Peroxisome Proliferator-Activated Receptor-γ (PPARγ plays a key role in shaping the metabolic response of CGNPs to Shh stimulation in a manner that persists after medulloblastoma formation [8]. The investigators show that E2F1 up-regulates PPARγ and that PPARγ in turn causes up-regulation of key glycolytic enzymes, Hexokinase 2 (Hk2) and Pyruvate Kinase M2 (PkM2), and of the glucose transporter Glut4. Inhibiting PPARγ in medulloblastoma-bearing ND2:SmoA1 mice decreased the expression of both Hk2 and PkM2, and reduced tumor glucose uptake, measured in vivo by ^{18}FDG-PET scan. Similar to the inhibition of CDK and FASN, PPARγ inhibition reduced the rate of tumor growth and extended survival [8]. These findings further demonstrate that the proliferative and metabolic functions of neural progenitors are interconnected and jointly become subverted in medulloblastoma tumorigenesis. Moreover, as shown in Fig. 1, this investigation demonstrates that changes in lipid and carbohydrate metabolizing enzymes occur in concert, jointly regulated by common intracellular signals.

A non-biased metabolomic analysis determined the functional significance of Shh-mediated changes in the expression of metabolic enzymes. CGNPs were explanted into media with or without Shh and changes in media metabolite concentrations were measured over time. Shh increased lactate production and glucose utilization of CGNPs, without causing additional changes in nutrient utilization and metabolite production. Continuous, real-time measurement of media oxygen content showed that Shh did not increase the CGNP oxygen consumption rate, and that Shh-treated CGNPs retained significant unused capacity for mitochondrial respiration. In vivo studies, including quantitative measurement of ^{18}FDG uptake and MR spectroscopy, further showed that glucose utilization and lactate production in the cerebellum were highest during the period of CGNP proliferation. ^{18}FDG-PET also demonstrated that the glycolytic phenotype of CGNPs is preserved in ND2:SmoA1 medulloblastoma. These studies confirmed that Shh signaling increased aerobic glycolysis in CGNPs and that Shh-driven medulloblastomas inherited the metabolic phenotype of their progenitor cells of origin [9].

Importantly, these studies revealed that hexokinase isoforms Hk1 and Hk2 were expressed in mutually exclusive domains in the postnatal brain, defined by the presence or absence of proliferating progenitors. While proliferating CGNPs up-regulated Hk2,

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