THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 289, NO. 33, pp. 22554–22556, August 15, 2014

Thematic Minireview Series on Phospholipase D and Cancer

Published, JBC Papers in Press, July 2, 2014, DOI 10.1074/jbc.R114.593137

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Phospholipase D (PLD) signaling plays a critical role in cell growth and proliferation, vesicular trafficking, secretion, and endocytosis. At the cellular level, PLD and its reaction product, phosphatidate, interact with a large number of protein partners that are directly related to the actin cytoskeleton and cell migration. Cancer invasion and metastasis rely heavily on cellular motility, and as such, they have put PLD at center stage in cancer research. This minireview series highlights some of the molecular mechanisms that provide evidence for the emerging tumorigenic potential of PLD, the role of the microenvironment, and putative connections with inflammation. PLD represents a potential target for the rational development of therapeutics against cancer and other diseases.

PLD[3] catalyzes the conversion of phosphatidylcholine to PA and choline. The enzyme reaction was first identified from carrots in 1947 by Hanahan and Chaikoff (1, 2), and indeed, most of the early work on the biochemistry of PLD was performed with the enzyme from plants (3). The existence of PLD in mammals was not discovered until 1973 (4). From the mid-1980s to the early 1990s, it became apparent that PLD played a major role in lipid signaling by generating PA, which was then converted to diacylglycerol for the activation of PKC (5). Interest in PLD intensified with the observations that PA itself governed several physiological processes that include cell growth and proliferation, vesicular trafficking, secretion, and endocytosis (6–12).

Studies on PLD were challenged with serious difficulties due to limited knowledge of its regulation on a molecular level, as well as the difficulty of purifying the enzyme from natural sources. However, the PLD field received a strong boost in 1994 when Wang *et al.* (13) identified the PLD gene from castor bean. This seminal contribution led to the identifications of orthologous genes from yeast (14) and mammals (15–17). The field also expanded when the action of PLD was implicated in Parkinson and Alzheimer diseases, as well as in several cancers.

Apart from generating one of the major lipid second messengers, PA, PLD interacts with a large number of protein partners, some of which are directly related to the actin cytoskeleton, which is involved with the protein machinery responsible for cell adhesion and migration (18). Precisely for this role, certain pathologies that rely on cell migration, such as cancer metastasis, have put PLD at center stage in cancer research. High levels of PLD activity are reported in a variety of cancers, such as breast, gastric, colorectal, and lung (19–22) cancers. Further, radiation in combination with PLD inhibition (specific for both PLD1 and PLD2) has been shown to be an efficient way to improve radiosensitivity of breast cancer cell lines and in animal models (23–26).

Recent studies with animal models have indicated that PLD is integral to breast cancer progression by increasing tumor growth and cell invasion (27–29). Significant expansion of the PLD field is expected in the near future considering the availability of new genetic, biological, and chemical tools, particularly PLD knock-out mice and new isoform-specific inhibitors that are being used to tease apart the contributing roles of the different PLD isoforms in cancer and other disease states (30–34).

Based on these advances, it is timely to review the multiple roles of PLD in cancer. This minireview series discusses the tumorigenic potential of PLD both *in vitro* and *in vivo*, the role of the microenvironment, and the emerging connections with inflammation. Although current studies are paving the way to a better understanding of PLD as a major drug target candidate in cancer therapy, it is still not clear what effects off-target PLD inhibition might have or how impeding PA formation might affect cell physiology as a whole because PA is a major component of both lipid metabolism and cell signaling. Insights obtained in the studies presented herein will help further investigations of the field.

In the first minireview, "Phospholipase D in Cell Signaling: From a Myriad of Cell Functions to Cancer Growth and Metastasis," Julian Gomez-Cambronero (35) focuses on PLD2, a protein that functions as both a PLD enzyme and a guanine nucleotide exchange factor. This review discusses how PLD2 acts as an intracellular signaling protein with roles in cell migration through actin polymerization and protein-protein associations with a wide network of molecules, including signaling kinases. Additionally, this minireview discusses the roles of PLD in cell transformation, tumor growth, and cancer metastasis.

The second minireview, "Cellular and Physiological Roles for Phospholipase D1 in Cancer" by Zhang and Frohman (36), highlights the generation and utilization of PLD1-deficient mice and development of small molecule PLD-specific inhibitors to define the roles for PLD1 in cancer. The review also summarizes recent findings regarding PLD1 functions in angiogenesis and metastasis.

The third minireview, "Functional Regulation of Phospholipase D Expression in Cancer and Inflammation" by Kang *et al.* (37), discusses the relationships between PLD dysregulation

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[3] The abbreviations used are: PLD, phospholipase D; PA, phosphatidate; mTOR, mammalian target of rapamycin.

and human cancer and inflammatory diseases that have spurred recent interest in therapeutics that target PLD function. This review summarizes progress made on the use of small molecule PLD inhibitors for the suppression of PLD expression and for the attenuation of PLD activity.

In the fourth and last minireview, "Phospholipase D and the Maintenance of Phosphatidic Acid Levels for Regulation of mTOR", Foster *et al.* (38) highlight the coordinated maintenance of intracellular PA levels that regulate mTOR signaling, as stimulated by growth factors and nutrients. This minireview discusses how cells compensate for the loss of one PA-generating system with the activation of an alternate system to generate PA. Regulating PA levels has important implications for cancer cells that are dependent on PA and mTOR activity for survival.

The PLD field lost two of its major contributors in 2008 when both Dennis Shields (Albert Einstein College of Medicine) and Mordechai Liscovitch (Weizmann Institute of Science Rehovot) passed away. Dr. Shields focused on the biochemical mechanisms of regulated secretion from the Golgi, whereas Dr. Liscovitch focused on cancer cell proliferation and survival. Their achievements provided the foundation of the enormous advances that the PLD field has experienced over the past 15 years. They will be remembered by those of us who were lucky to have met them, as superb researchers, teachers, and individuals with great humanity and dedication to their students. This minireview series is dedicated as a memorial to these outstanding investigators.

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