Cellular Multitasking

Protein Moonlighting in Cancer

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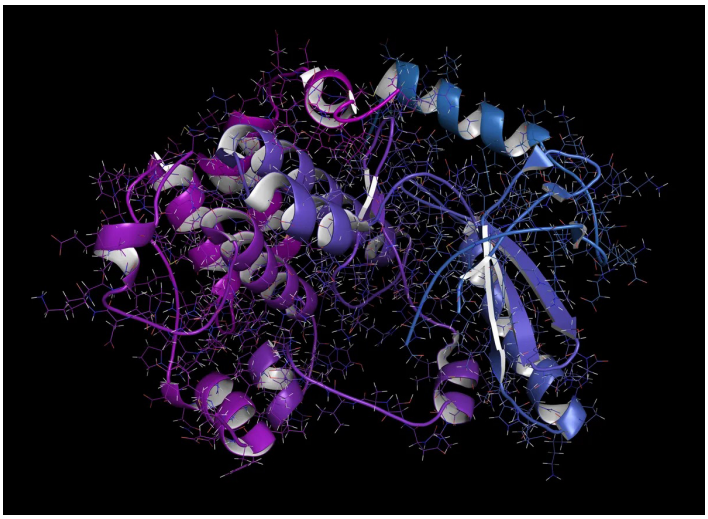


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Introduction

Cancer is a group of diseases characterized by uncontrolled cell division. Within cancer cells, the coordinated activities of molecules allow for adaptation to hostile environments and often confer resistance to treatment (Housman et al., 2014). Among these molecules are proteins, whose diverse roles play out all across the cell, from structural support and transport to signaling and metabolism (Morris et al., 2022). Traditionally, individual proteins have been viewed as having single, well-established functions, in line with George Beadle and Edward Tatum's well-known 'one gene-one enzyme' hypothesis (Beadle & Tatum, 1941). In the 1980s, protein multifunctionality was suggested when molecular biologists Joram Piatigorsky and Graeme Wistow noticed that some crystallins, structural proteins found in vertebrate eyes, also function as enzymes. Initially described as gene sharing, this phenomenon, where proteins can perform several unrelated functions, has become known as protein moonlighting (Huberts & van der Klei, 2010). Moonlighting proteins have recently emerged as potential targets in cancer therapy.

Many moonlighting proteins help maintain homeostasis by serving as receptors, signaling molecules, or gene expression regulators (Jeffery, 2017). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), for example, is a known enzyme in glycolysis, but can also influence gene expression by affecting the stability of DNA and gene products (Adamo et al., 2021). When cellular iron levels are low, GAPDH can also localize to the cell surface to act as a receptor for a protein that binds iron (Kumar et al., 2011).

However, moonlighting proteins can also contribute to disease – more than 78% of known moonlighting proteins are associated with human pathologies. Changes in the cellular environment during disease development can activate the pathogenic moonlighting functions of moonlighting proteins (Huerta et al., 2023). For instance, GAPDH can also support hepatitis C infection of host cells by aiding in viral replication, and in many bacteria, glycolytic enzymes secreted to the cell surface support bacterial colonization of host cells (Raj et al., 2017; Rodríguez-Saavedra et al., 2021). Currently, cancer therapies commonly target proteins based on a single pathogenic function (Kędzierska & Bańkosz, 2024). However, targeting moonlighting proteins may enhance therapies, as inhibition of one protein can inhibit several pathways that together promote cancer survival. Exploiting the versatility of moonlighting proteins may allow medicine to better overcome the resilience of cancer cells.

Moonlighting Proteins in Cell Growth and the Cell Cycle

Moonlighting proteins are emerging as important players in the cell cycle, whose dysregulation gives rise to cancer. In the cell cycle, moonlighting proteins help repair DNA, trigger cell death, and interact with other cell cycle proteins to modulate progression through the cell cycle (Han et al., 2017; Manickavinayaham et al., 2020). The autosomal recessive hypercholesterolemia (ARH) protein, for example, has a primary function aiding the uptake of cholesterol from blood. However, ARH has also been shown to interact with the centrosome, an organelle that is vital for the partitioning of genetic material among dividing cells. During cell division, centrosomes build fibers that attach chromosomes, and ARH localizes to these attachment sites, helping to carry out proper division (Lehtonen et al., 2008).

Moonlighting proteins can also influence the cell cycle by acting in signaling pathways, thereby influencing a cascade of events. The Krebs cycle enzyme fumarate hydratase (FH) is one such protein. FH is primarily known to convert fumarate to malate in the Krebs cycle, but it also appears to be able to act as a tumor suppressor via inactivation of a growth receptor. In this way, FH helps maintain regulated growth of cells by preventing aberrant signaling (Wang et al., 2023). There appears to be a complex network of protein interactions that helps preserve the integrity of dividing cells, making it consequential when the proteins adopt rogue behavior.

Protein Moonlighting Supports Cancer Survival

Single moonlighting proteins may each sustain cancer cell proliferation through activity in multiple processes, thus therapeutically targeting them may significantly compromise cancer's resilience. Moonlighting proteins can help cancer cells rewire their metabolism, escape cell death, and invade nearby cells while evading the immune system (Adamo et al., 2021). A hallmark of cancer cells is their preference for energy generation via the oxygen-independent process of glycolysis, even in oxygen-rich environments (Liberti & Locasale, 2016). Many glycolytic enzymes are moonlighting proteins, including pyruvate kinase M2 (PKM2), which drives the final step of glycolysis (Gao et al., 2022). PKM2 supports cancer metabolism by allowing glycolysis to continually be used for energy production, but its moonlighting role also allows it to interact with proteins involved in pathways regulating cell death. PKM2 can both drive anti-cell death signaling and

inhibit cell-death pathways, thus its overexpression in cancer more directly bolsters cancer cell survival (Chen et al., 2026). PKM2 may be indicative of the therapeutic potential of other moonlighting proteins in cancer, where the inhibition of one protein can inhibit several processes necessary for proliferation, increasing cancer cell vulnerability.

Beyond resisting death at its site of origin, cancer can spread to healthy tissues, posing great therapeutic challenges and causing the majority of cancer-related deaths (Gerstberger et al., 2023). Metastasis requires the degradation of the molecular network surrounding cells, or the extracellular matrix (ECM). One moonlighting protein involved in this process is enolase, a glycolytic enzyme that can moonlight as a cell-surface receptor for a protein called plasminogen. Bound plasminogen can be converted into the plasmin protein, which can degrade the ECM. Thus, by promoting the production of ECM-degrading proteins, enolase aids cancer metastasis (Huang et al., 2022). Metastasis is also supported by the moonlighting function of the IDO1 protein, because cancer cells must also escape immune detection to metastasize. The IDO1 protein has a known enzymatic function in a signaling pathway, but it can also act as a signaling molecule in pathways suppressing immune cell activity. This activity hinders the ability of the immune system to detect cancer cells, allowing cancer cells to invade healthy tissues (Adamo et al., 2021).

Cancer cells are sustained by a complex network of interactions involving moonlighting proteins. This offers an opportunity for more effective targeting of cancer, as multiple processes needed for cancer cell survival may fall upon inhibition of just one protein's activity.

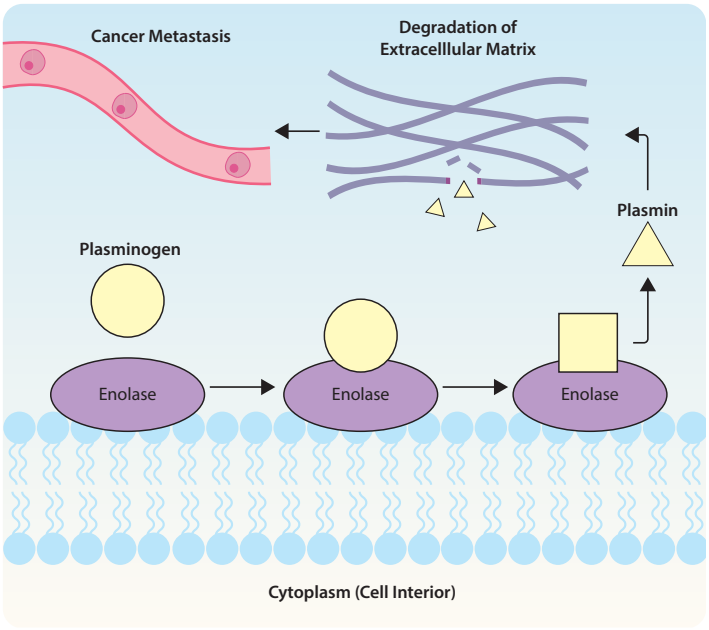


Figure 1. Enolase moonlighting supports cancer metastasis. (Figure courtesy of Krish Muniappan)

Targeting Moonlighting Proteins

Medicine has already made attempts to act on the understanding of protein moonlighting. Therapy against moonlighting proteins holds both promises and challenges. Inhibition of pathogenic moonlighting proteins may be very effective at sensitizing cancer cells, as several abnormal activities can be targeted at once; however, this approach may lead to several side effects as the normal functions of the proteins may also be disrupted.

Challenges persist in targeting moonlighting proteins clinically, because some drugs may not produce an impactful response while others may produce undesired side effects. The drug epacadostat was developed against IDO1 and failed higher clinical trials when it was not sufficiently effective in cancer patients, possibly because the drug unintendedly led to further suppression of immune cells (Adamo et al., 2021). Additionally, as many existing therapies do, targeting a protein's moonlighting function may unavoidably affect its primary role, harming normal functions alongside pathological ones. Still, cancer medicine can benefit from targeting moonlighting proteins, as the activity of proteins enables the development of drug resistance that drives 90% of chemotherapy failures (Mansoori et al., 2017).

Laboratory investigations on targeting moonlighting proteins have revealed several new potential drugs and therapies. For example, the glycogen synthase kinase-3 (GSK-3) protein has a primary function in regulating the metabolism of sugars and several moonlighting roles in affecting signaling events associated with cell proliferation (Siddavaram Nagini et al., 2018). Tideglusib has been developed as a molecule to inhibit GSK-3, and has shown effectiveness in killing neuroblastoma cells that drive pediatric brain cancers (Mathuram et al., 2016). There have also been efforts to target enolase by making antibodies that bind and inhibit cell-surface enolase, leading to a reduction in metastases (Principe et al., 2015). Targeting moonlighting proteins alongside administration of other treatments may help medicine overcome the adaptability of cancer cells, making treatments more effective for more cancers and patients.

Conclusion

Cancer cells display remarkable resilience that complicates their biology and cancer medicine. The hostile molecular environments that cancer cells face, along with the increased energy demand and upregulated metabolism within the cells, make adaptation necessary for their survival. Moonlighting proteins are particularly useful to cancer cells in this regard, enabling cancer cells to perform more processes without having to produce new proteins. Protein moonlighting allows for greater optimization of energy use, supporting cell growth and division, as well as stronger resistance against cellular stress, enhancing cell survival. Moonlighting proteins therefore bolster the resilience of cancer cells, instilling in them significant therapeutic promise.

Cancer cells are dynamic and strategic, yet the processes that allow them these qualities make them vulnerable. Protein moonlighting is one such process, and probing its potentials further could uncover more strategic therapeutic targets, ultimately improving outcomes in cancer medicine.

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