

CD11b⁺F4/80⁺ macrophages and CD11c⁺MHC-II⁺ DCs in the TME and periphery. To study myeloid cell-expressed PD1 in myelopoiesis and tumor growth, the authors generated myeloid cell-specific PD1-deficient mice. These mice were equally resistant to tumor growth as global PD1-deficient mice. Similar to global PD1-deficient mice, myeloid cell PD1-deficient mice presented with fewer MDSCs and more macrophages and DCs in the TME. This immunogenic skew in myeloid lineage commitment was associated with increased IRF8, a major transcription factor implicated in myelopoiesis. The work of Strauss *et al.* is consistent with previous studies suggesting that downregulation of IRF8 by MDSC-inducing factors, such as G-CSF, shifts myelopoiesis towards MDSCs in the TME [7]. G-CSF stimulation of PD1-deficient MPCs leads to enhanced activation of the ERK1/2, mTORC1, and STAT1 pathways, which are known to promote immunogenic myeloid differentiation. However, more effort is needed to elucidate how PD1-deficient MPCs may be transcriptionally programmed via IRF8.

Strauss *et al.* also defined metabolic pathways involved in the differentiation of PD1-deficient MPCs towards a monocyte-macrophage myeloid lineage. Consistent with their *in vivo* data, PD1-deficient MPCs effectively differentiated into Ly6C⁺ monocytic cells (macrophage and/or DC precursors) when cultured with cytokines that skew myelopoiesis in the TME [e.g., G-CSF, GM-CSF, and/or interleukin-6 (IL-6)]. This push towards a monocytic lineage was associated with an increased bioenergetic profile and higher cholesterol activity in PD1-deficient MPCs. Indeed, others have shown that cholesterol promotes the expansion of myeloid cells and the differentiation of macrophages and DCs [8].

Strauss *et al.* emphasize the differential importance of PD1 within myeloid cells versus T cells in antitumor immunity. Myeloid cell-intrinsic PD1 signaling appears critical to

alter myelopoiesis during cancer, because the skew in myeloid cell fate commitment from MDSCs to antitumor macrophages and DCs was not observed in T cell-specific PD1-deficient mice. The authors also linked this enhanced myelopoiesis in myeloid cell PD1-deficient mice with improved antitumor T cell function, including increased frequency of interferon (IFN)- γ /IL-17-producing effector memory CD8⁺ T cells. Despite some tumor-promoting effects of IL-17 [9], IL-17-producing CD4⁺ Th17 and CD8⁺ T cells have antitumor functions due to their polyfunctional cytokine profiles (including IFN- γ and stem-like features) [10]. In fact, Strauss *et al.* directly showed that PD1-deficient DCs from tumor-bearing mice better induced IFN- γ -secreting CD4⁺ and CD8⁺ T cells. Overall, their finding that tumor growth is better controlled in myeloid cell-specific PD1 deficient mice versus T cell-specific PD1-deficient mice underscores the crucial role of myeloid cell PD1 in antitumor immunity. This is striking given that PD1 cancer studies have thus far predominantly focused on T cell PD1 [2]. Interestingly, the data from Strauss *et al.* also suggest that improved T cell responses due to PD1 blockade are possibly mediated indirectly via PD1 signaling within myeloid cells.

Consistent with animal models of global and myeloid cell-specific PD1 deletion, anti-PD1 treatment decreases MDSCs while increasing monocytes and DCs in the TME, suggesting that blocking PD1 could work partially by correcting a tumor-promoting shift in myelopoiesis during cancer. Overall, this exciting work adds a new perspective on the key contribution of myeloid-intrinsic PD1 signaling to immune evasion in cancer. Immune phenotyping of TME myeloid cell populations, including their PD1 expression, may be useful as a prognostic biomarker for anti-PD1 therapies. Beyond direct effects on T cells, approaches for PD1 blockade combination therapies should consider synergy with myeloid cell biology, such as agents that promote myeloid cell immunogenicity and recruitment. Given that PD1 is

expressed on many types of myeloid cell, human PD1 function on specific myeloid cell subsets in cancer and other contexts awaits further discoveries.

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References

- Gajewski, T.F. *et al.* (2013) Innate and adaptive immune cells in the tumor microenvironment. *Nat. Immunol.* 14, 1014–1022
- Baumeister, S.H. *et al.* (2016) Coinhibitory pathways in immunotherapy for cancer. *Annu. Rev. Immunol.* 34, 539–573
- Riella, L.V. *et al.* (2012) Role of the PD-1 pathway in the immune response. *Am. J. Transplant.* 12, 2575–2587
- Garo, L.P. *et al.* (2019) Smad7 Controls immunoregulatory PDL2/1-PD1 signaling in intestinal inflammation and autoimmunity. *Cell Rep.* 28, 3353–3366
- Strauss, L. *et al.* (2020) Targeted deletion of PD-1 in myeloid cells induces antitumor immunity. *Sci. Immunol.* 5, eaay1863
- Awad, R.M. *et al.* (2018) Turn back the TIME: targeting tumor infiltrating myeloid cells to revert cancer progression. *Front. Immunol.* 9, 1977
- Waight, J.D. *et al.* (2013) Myeloid-derived suppressor cell development is regulated by a STAT/IRF-8 axis. *J. Clin. Invest.* 123, 4464–4478
- Tall, A.R. and Yvan-Charvet, L. (2015) Cholesterol, inflammation and innate immunity. *Nat. Rev. Immunol.* 15, 104–116
- Murugaiyan, G. and Saha, B. (2009) Protumor vs antitumor functions of IL-17. *J. Immunol.* 183, 4169–4175
- Bowers, J.S. *et al.* (2017) Th17 cells are refractory to senescence and retain robust antitumor activity after long-term *ex vivo* expansion. *JCI Insight* 2, e90772

Forum

Towards the Microbial Production of Plant-Derived Anticancer Drugs

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Many of the plant-derived compounds used in chemotherapies are

currently produced by semisynthesis, which results in limited supplies at exorbitant market prices. However, the synthetic biology era, which began ca 15 years ago, has progressively yielded encouraging advances by using engineered microbes for the practical production of cheaper plant anticancer drugs.

Plant-Derived Anticancer Drugs: Low Supply, High Cost

Plants represent a seemingly inexhaustible source of biologically active compounds. Some of these have been essential components of the anticancer therapeutic arsenal for many years [1]. Unfortunately, most of these chemicals naturally accumulate in the plant in only very small quantities and their complex structures render total chemical syntheses highly impractical. As a consequence, although pharmaceutical companies have optimized the extraction of precursors and developed semisynthetic processes for most of the valuable plant drugs currently used in the clinic, these active compounds usually have a high cost of production. In an attempt to circumvent these production limits, alternative strategies based on metabolic engineering have been widely explored over the past 15 years. Taking inspiration from the pioneering success of the antimalarial semisynthetic artemisinin [2], microbial cell factories are increasingly developed for the production of plant-derived drugs. Numerous examples of genetically engineered bacteria and/or yeasts have emerged as suitable production hosts in the past decade. In this context, the plant genes that are heterologously expressed in these microbes allow *de novo* synthesis of the expected drugs. This can be achieved either through the derivatization of endogenous metabolites from these microbes or by the biotransformation of a cheap and abundant precursor that can be exogenously supplied to the microbe culture medium. In this Forum, we discuss the current status of this field by describing

several major advances recently achieved for prominent classes of plant-derived anticancer drugs.

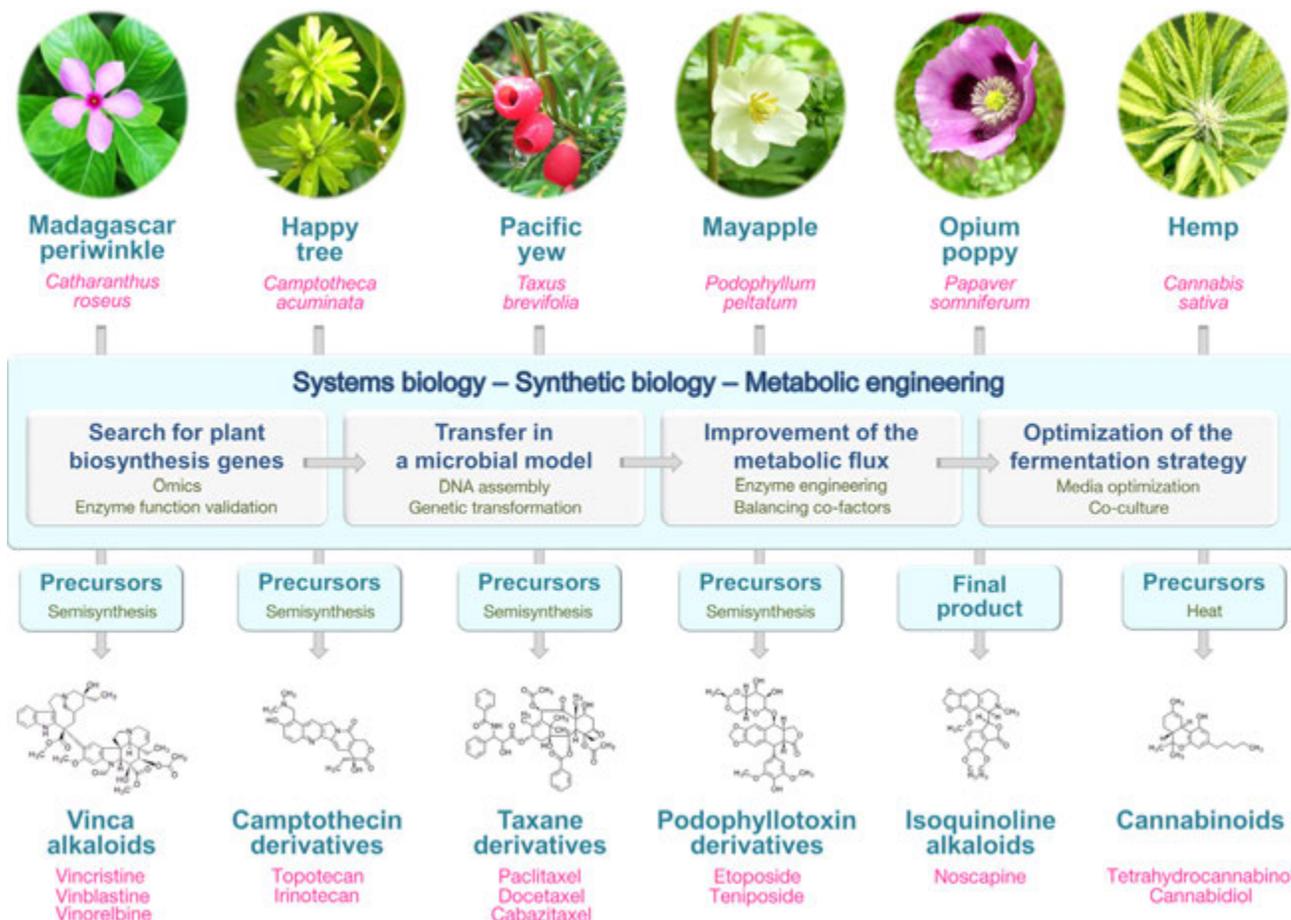
Monoterpene Indole Alkaloids (MIAs) from Apocynaceae

Several MIAs found in Apocynaceae, in particular those from the Madagascar periwinkle (*Catharanthus roseus*), are long known for their antimitotic activity and are still used for treating various types of cancers [1]. This series of vinca alkaloids includes the natural compounds vincristine and vinblastine as well as the 'non-natural' derivatives vinorelbine, vindesine, and vinflunine. All of these compounds are powerful chemotherapy medications against various forms of leukemia, lymphoma, and solid tumors. After FDA approval in 1963, these anticancer MIAs have been produced by semisynthesis; that is, partial chemical synthesis using compounds isolated from natural sources as starting materials. Specifically, the production of these compounds relies on the coupling of the precursor monomers vindoline and catharanthine to generate the biologically active vinca-type alkaloids, with the first chemical procedure for coupling vindoline and catharanthine to form vinblastine described in 1974. Because both vindoline and catharanthine monomers accumulate in the aerial parts of *C. roseus* in only small quantities, the current low-yield production of vinca alkaloids leads to exorbitant market prices that can exceed several tens of millions of dollars per kilogram for vincristine [3]. Thus, by the end of the 1970s, various research groups began to search for enzymes involved in MIA biosynthetic pathways with the goal of using metabolic engineering to improve the production of vindoline and catharanthine monomers. Forty years later, almost all of the genes involved in MIA synthesis have been characterized in *C. roseus* [4]. Moreover, some proof-of-concept studies have emerged recently through the development of yeast strains

hosting numerous periwinkle biosynthetic genes for the engineered production of anticancer MIA precursors (Figure 1) [5]. The MIA intermediates strictosidine and nepetalactone have been thus successfully produced *de novo* in engineered yeast strains, and vindoline has been produced by feeding the intermediate tabersonine to engineered yeasts as well. However, the titers of these engineered yeast strains for *de novo* production remain at submilligram levels, indicating that substantial optimization is required to commercialize these production processes. Considerable effort is being made in this area, and given the successes with the production of other plant-derived compounds in yeast, we are optimistic that microbial cell factories will be soon available for participation in lower-cost production processes of MIA-based anticancer formulations. Undoubtedly, such advances on vinca alkaloids will also pave the way for implementing microorganisms producing precursors of biosynthetically related topotecan and irinotecan, the highly potent antitumor MIAs, derived from the happy tree (*Camptotheca acuminata*) camptothecin (Figure 1) [6].

Taxane Derivatives from the Pacific Yew

Paclitaxel (Taxol), docetaxel (Taxotere), and cabazitaxel represent another important class of plant-derived compounds extensively used in chemotherapy, notably for AIDS-related Kaposi sarcoma and various solid tumors [1]. These compounds belong to the taxane family, which are diterpenoids produced by yews (*Taxus* genus) (Figure 1). Like MIAs, taxanes display complex structures that make bulk chemical synthesis impossible in a cost-effective manner. Notably, the biotechnological production of taxane derivatives has been successful in plant cell cultures since the 2000s [7]. While this type of production has been a commercial success, additional effort



Trends in Cancer

Figure 1. Developing Microbial Synthetic Biology for the Production of Plant-Derived Anticancer Drugs. Schematic representation of the global strategy for implementing the production by engineered microbes of monoterpenoid indole alkaloids of the Madagascar periwinkle, yew taxane-type terpenoids, mayapple lignans, poppy isoquinoline alkaloids, and hemp cannabinoids. The development process relies on a combination of systems biology, synthetic biology, and metabolic engineering strategies. Usually, this begins with the search for biosynthetic genes in the medicinal plant. Once identified and characterized, these genes are transferred into a microbial host by genetic transformation. Improvement of the metabolic flux and optimization of the fermentation strategy must be rationally achieved to improve the production yields of these engineered microbes, whether as a precursor or a final product.

has been made to engineer taxane biosynthesis, especially by creating bacterial or yeast strains and bacterial/yeast consortia that produce upstream precursors [8,9]. However, many hurdles remain in implementing a sustainable supply of taxane using microorganisms. Most importantly, many biosynthetic enzymes from the yew taxane biosynthetic pathway have not been discovered and this clearly prevents the heterologous reconstitution of the complete pathway. Nevertheless, using the same omics-based global strategies as utilized for the MIA pathway [4],

the identification of these enzymes could be achieved in the foreseeable future.

Lignan Derivatives from the Mayapple Podophyllotoxin

Etoposide and teniposide are lignan derivatives widely used in chemotherapies for testicular cancer, lung cancer, lymphoma, leukemia, neuroblastoma, and ovarian cancer [1]. Both compounds are currently supplied by chemical modifications of the podophyllotoxin skeleton, a lignan that accumulates at low levels in the roots of the mayapple (*Podophyllum peltatum*)

(Figure 1). The restricted access to this endangered medicinal plant, which has been overharvested, has recently caused supply disruption. A few years ago, a huge leap forwards was accomplished with the identification and characterization of the key missing enzymes for podophyllotoxin synthesis *in planta* [10]. The proof of concept of heterologous production of this precursor was provided by transferring the whole set of mayapple biosynthetic genes into tobacco plants allowing, in turn, the conversion of a common plant natural precursor into podophyllotoxin analogs. Since the

biosynthetic pathway of these lignans is shorter than that of vinblastine and taxanes described above, we anticipate that the development of practical microbial cell factories for these important anticancerous lignans can be rapidly achieved.

Benzylisoquinoline Alkaloids from Opium Poppy

Opium poppy (*Papaver somniferum*) is a source of potent pharmaceutical compounds showing a wide variety of activities. Among these complex structures related to the family of benzylisoquinoline alkaloids, which include the opioids, noscapine exhibits exciting anticancer potential but is accumulated only in low quantities *in planta*. In this context, Li *et al.* [11] have recently achieved outstanding *de novo* production of noscapine in yeast by recreating a biosynthetic pathway comprising over 30 enzymes from plants, bacteria, mammals, and yeast (Figure 1). Although noscapine production titers remain low (2.2 mg/l of yeast culture), this work opens unprecedented perspectives for an upcoming development of production platforms for anticancer opioids and illustrates that sourcing enzymes from distinct organisms is often necessary for efficient microbial production.

Cannabinoids from Hemp

Although displaying some *in vitro* antiproliferative and proapoptotic effects, cannabinoids from hemp (*Cannabis sativa*), mainly tetrahydrocannabinol and cannabidiol, are not clinically used as anticancer medicines. However, the use of these compounds by cancer patients is currently booming due to their capacity to reduce pain caused by cancer and/or chemotherapy. In addition, these cannabinoids have been approved as antiemetics, anticachexics, analgesics, or antispastic medicines by several regulatory agencies [12]. However, cannabinoid synthesis in hemp can be highly variable *in planta* and the complex structures limit chemical synthesis approaches, thus requiring the development of complementary processes of supply to meet the demand.

In this regard, Luo *et al.* [13] recently performed the complete biosynthesis of several cannabinoids in yeast (Figure 1). Although some strain improvements are required to reach production at an industrial scale, this work provided the proof of concept that engineered microbes can efficiently supply cannabinoids for pain management during cancer. Furthermore, by exploiting enzymes that have the capacity to metabolize different substrates, new-to-nature cannabinoid analogs were also produced in this study, thereby opening further possibilities for the production of new, related structures with novel pharmaceutical properties.

Concluding Remarks

Synthetic biology has driven major advances in the development of microbial cell factories that produce natural products since the beginning of the 2000s. A few recent works have demonstrated the feasibility of production platforms for several families of plant-derived anticancer drugs – that is, opioids, cannabinoids, and alkaloids – and these successes suggest that we can anticipate the same for taxanes and lignans (Figure 1). However, although the development of yeast strains at the laboratory scale represents a major step we can celebrate, much work remains to be performed before we reach industrial-scale production of strains that can be used for commercialization. These bottlenecks include: (i) low gene expression, which could be solved by increasing gene copy number using CRISPR/Cas9 technology; (ii) low metabolic fluxes that could be optimized by increasing the production of enzyme co-factors in the microbial host; (iii) improving host tolerance to the produced drug by the timely induction of transgene expression and/or product capture during yeast culture; and (iv) compensating for slow growth of the engineered microbe by optimization of the fermentation conditions, for example (Figure 1) [14]. Importantly, addressing these drawbacks will benefit all of the aforementioned metabolic engineering

approaches. These advances will also pave the way for the future production of new cytotoxic natural products from other biological sources, in particular those identified in marine organisms [15].

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References

1. Srivastava, V. *et al.* (2005) Plant-based anticancer molecules: a chemical and biological profile of some important leads. *Bioorg. Med. Chem.* 13, 5892–5908
2. Paddon, C.J. and Keasling, J.D. (2014) Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. *Nat. Rev. Microbiol.* 12, 355–367
3. Gordon, N. *et al.* (2018) Trajectories of injectable cancer drug costs after launch in the United States. *J. Clin. Oncol.* 36, 319–325
4. Caputi, L. *et al.* (2018) Missing enzymes in the biosynthesis of the anticancer drug vinblastine in Madagascar periwinkle. *Science* 360, 1235–1239
5. Brown, S. *et al.* (2015) *De novo* production of the plant-derived alkaloid strictosidine in yeast. *Proc. Natl. Acad. Sci. U. S. A.* 112, 3205–3210
6. Sadre, R. *et al.* (2016) Metabolite diversity in alkaloid biosynthesis: a multilane (diastereomer) highway for camptothecin synthesis in *Camptotheca acuminata*. *Plant Cell* 28, 1926–1944
7. Yukimune, Y. *et al.* (1996) Methyl jasmonate-induced overproduction of paclitaxel and baccatin III in *Taxus cell suspension cultures*. *Nat. Biotechnol.* 14, 1129–1132
8. Zhou, K. *et al.* (2015) Distributing a metabolic pathway among a microbial consortium enhances production of natural products. *Nat. Biotechnol.* 33, 377–383
9. Biggs, B.W. *et al.* (2016) Overcoming heterologous protein interdependency to optimize P450-mediated Taxol precursor synthesis in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 113, 3209–3214
10. Lau, W. and Sattely, E.S. (2015) Six enzymes from mayapple that complete the biosynthetic pathway to the etoposide aglycone. *Science* 349, 1224–1228

11. Li, Y. *et al.* (2018) Complete biosynthesis of noscapine and halogenated alkaloids in yeast. *Proc. Natl. Acad. Sci. U. S. A.* 115, E3922–E3931
12. Byars, T. *et al.* (2019) Using cannabis to treat cancer-related pain. *Semin. Oncol. Nurs.* 35, 300–309
13. Luo, X. *et al.* (2019) Complete biosynthesis of cannabinoids and their unnatural analogues in yeast. *Nature* 567, 123–126
14. Choi, K.R. *et al.* (2019) Systems metabolic engineering strategies: integrating systems and synthetic biology with metabolic engineering. *Trends Biotechnol.* 37, 817–837
15. Blunt, J.W. *et al.* (2018) Marine natural products. *Nat. Prod. Rep.* 35, 8–53

Forum

Lipid in Renal Carcinoma: Queen Bee to Target?

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Clear cell renal cell carcinoma (ccRCC) is the most common renal cancer subtype, characterized by a lipid storage phenotype. We found that carnitine palmitoyltransferase 1A (CPT1A), the rate-limiting enzyme of mitochondrial fatty acid (FA) transport, is repressed by hypoxia-inducible factors (HIFs), reducing FA oxidation (FAO). Altering lipid metabolism may be a new therapeutic avenue in ccRCC.

Renal cell carcinoma (RCC) is common in the USA, estimated to account for 73 820 incidences and 14 770 deaths in 2019 [1]. There are four main subtypes of RCC, and the most common form is ccRCC which makes up approximately 70% of all renal malignancies [2]. While surgery remains the standard of care for patients diagnosed with early-stage ccRCC, approximately 30% of patients eventually progress to metastatic disease which has an extremely dismal 5-year survival rate [2]. ccRCC is refractory to conventional cytotoxic chemotherapy and radiotherapy, and the mechanisms of resistance remain unclear.

More than 92% of ccRCC cases are sporadic and are canonically associated with biallelic inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene located on chromosome 3p [2]. The remaining cases develop, in a hereditary fashion, as part of VHL disease, with VHL mutation in the family history [3]. The product of the VHL gene functions most notably as part of the ubiquitin-mediated proteasomal degradation system, targeting the HIF α subunits for turnover [3]. Under normoxic conditions, HIF α are ubiquitinated and degraded; while under hypoxic conditions HIF α are stabilized and function as transcriptional activators in an elaborate cell stress response system [2]. In ccRCC, due to large chromosome 3p deletions, epigenetic silencing, and/or an inactivating point mutation of VHL protein, the HIF α subunits are stabilized irrespective of oxygen content [3]. This results in constitutively active HIF signaling and increases the transcription of hypoxia-associated genes, such as vascular endothelial growth factor (VEGF), which stimulates tumor angiogenesis [3]. This has been indicated to be one of the main oncogenic pathways for development of ccRCC, and most of the therapeutic strategies developed in the past two decades have been aimed at targeting VEGF with drugs, such as sunitinib, pazopanib, or axitinib [2]. In ccRCC, HIF1 α , but not HIF2 α , activity is lost by homozygous deletion of the *HIF1 α* locus and is tumor suppressive; despite having many overlapping target genes with HIF1 α , HIF2 α is shown to be overexpressed in ccRCC and has tumorigenic potential [2].

Lipid Metabolism in ccRCC

As the name itself implies, ccRCC cells are characterized histologically to have ‘clear’ cytoplasm, as the intracellular accumulation of lipids and glycogen is removed during the standard histological preparations [2]. A report by Tun *et al.* demonstrated that ccRCC displays a switch in adipogenic gene signatures when normal renal epithelial cells undergo

transdifferentiation during their transition into tumor [4]. Lipid reprogramming in ccRCC is a well-documented phenomenon, yet the significance of this process is still not well understood.

FA metabolism has two components: an anabolic process where various nutrients are converted into metabolic intermediates in order to generate FAs for energy sources, maintaining cell membrane, and mediating signaling; and a catabolic process in which FAs are transported into mitochondria by the carnitine transport system for β -oxidation in order to regenerate acetyl-CoA for entry into the tricarboxylic acid (TCA) cycle as well as the reducing equivalents for ATP production [5]. Abnormal cancer metabolism can result in the loss of balance between these two reactions, resulting in lipid accumulation in cancer cells. *De novo* lipogenesis (DNL) has been widely studied as the mechanism of lipid storage in many lipid-associated cancers, including ccRCC, glioblastoma, breast, and colorectal cancers [2,5]. Proliferating cancer cells rely on biosynthetic intermediates, such as FAs, to support increased metabolic needs for membrane and organelle formation [3].

Lipid Droplets in ccRCC

Lipid droplets (LDs), which are a prominent phenotype of ccRCC, are composed mainly of triglycerides (TGs) and cholesterol esters (CEs) [6]. Lipidomic studies have demonstrated that both TGs and CEs exist at higher levels in ccRCC compared with normal kidney tissues [7]. TGs consist of a glycerol backbone linked with three FAs, which can be of various chain lengths and degrees of saturation.

Although lipid accumulation in ccRCC has been described for many decades, the beneficial roles of LDs for tumorigenesis are still under debate. Some have suggested that storage of esterified FAs in LDs can protect the cells from lipotoxicity