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REVIEW

Neutrophil extracellular traps and metabolic reprogramming in renal cell carcinoma: implications for tumor progression and immune-based therapeutics

Asif Shahzad[1]*, Yueli Ni[1]*, Zhuoran Teng[1]*, Wenjing Liu[1]*, Honggang Bai[2]*, Yijian Sun[1], Kun Cui[1], Qiuxin Duan[1], Xiangjie Liu[1], Zhe Xu[1,3], Jinshan Zhang[1], Jiaojiao Xia[1], Rong Che[3], Ting Guo[4], Zhe Yang[5], Qiao Zhang[1]

[1]Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Kunming Medical University, Kunming 650500, China; [2]Department of Clinical Laboratory, The Second Hospital of Jingzhou, Jingzhou 434000, China; [3]School of Basic Medical Sciences, Qujing Medical College, Qujing 655000, China; [4]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Division of Gastrointestinal Cancer Translational Research Laboratory, Peking University Cancer Hospital & Institute, Beijing 100142, China; [5]Department of Pathology, The First Affiliated Hospital of Kunming Medical University, Kunming 650032, China

ABSTRACT Neutrophil extracellular traps (NETs) are web-like structures of DNA and proteins that are released by activated neutrophils. While originally identified as antimicrobial defense mechanisms, NETs are now recognized as key modulators of tumor progression. NETs interact with the tumor microenvironment and metabolic pathways in renal cell carcinoma (RCC), which promotes immune evasion and metastasis. This review explores the interplay between NET formation and metabolic reprogramming in RCC, highlighting the implications for immunotherapy resistance and therapeutic targeting. NET-associated signaling, immunometabolism disruption, and current strategies to inhibit NETs in preclinical and clinical settings are discussed. Targeting NETs may represent a promising adjunct in RCC therapy, particularly when integrated with immune checkpoint blockade.

KEYWORDS Neutrophils; neutrophil extracellular traps; renal carcinoma cell; metabolic reprogramming; cancer immunity; therapeutic target

Introduction

Neutrophils are the most abundant subtype of white blood cell, constituting 50–70% of circulating leukocytes in humans and 10–25% in rodents[1]. Neutrophils are pivotal in the innate immune response, providing a first line of defense against pathogens, such as bacteria, viruses, and fungi[2]. Neutrophils are promptly activated and recruited to infection sites upon microbial or foreign invasion, where neutrophils neutralize threats *via* reactive oxygen species (ROS) production, phagocytosis, and degranulation[3]. In 2004 Brinkmann et al. identified a unique form of neutrophil activation involving the release of decondensed DNA decorated with granular proteins [neutrophil extracellular traps (NETs)][3]. These extracellular DNA structures have been shown to trap and neutralize pathogens effectively. Since the discovery of NETs extensive research over the past two decades has explored the physiologic and pathologic roles of NETs in immune-mediated inflammatory and metabolic disorders[4,5]. More recently, NETs have been implicated in the pathophysiology and progression of malignancies, with growing evidence highlighting a pro-tumorigenic role. NETs contribute to tumorigenesis by fostering an inflammatory tumor microenvironment (TME) and interacting with pro-tumorigenic pathways, including autophagy and inflammasome activation[6].

Renal cell carcinoma (RCC), commonly known as kidney cancer, is a condition with a rising incidence that frequently intersects with the domain of nephrology[7]. Despite the increasing prevalence of RCC, this intriguing and clinically significant disease often receives insufficient attention within

*These authors contributed equally to this work.
Correspondence to: Ting Guo, Zhe Yang and Qiao Zhang
E-mail: luckyguoting@bjmu.edu.cn, yangzhe@ydyy.cn and zhangqiao3@kmmu.edu.cn
https://orcid.org/0000-0003-0199-5639,
https://orcid.org/0000-0003-3961-5398 and
https://orcid.org/0000-0003-4588-6813
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nephrology training programs and is underrepresented in scientific and clinical discussions at national nephrology conferences[8,9]. While traditional cancer research has predominantly focused on mutations affecting growth-regulating pathways and the discovery of oncogenes, contemporary investigations, particularly in kidney cancer, have unveiled alterations in metabolic pathways composed of these same genetic factors, leading to tumor energetics and biosynthesis. This phenomenon, known as metabolic reprogramming, involves the adaptation of classical biochemical pathways to favor tumor' survival and proliferation. Patients with advanced RCC stage still have a poor prognosis despite advances in targeted and immunologic therapies with few options for systemic treatment and significant recurrence rates following locoregional therapies[10,11]. The TME is a complex milieu comprised of interactions among tumor cells, stromal components, and immune cells, which have pivotal roles in tumorigenesis, metastasis, and therapeutic resistance. Despite the emergence of targeted therapies and immune checkpoint inhibitors, the prognosis of patients with advanced RCC remains suboptimal. RCC exhibits a paradoxical immune phenotype that is characterized by high $CD8^+$ T cell infiltration yet a poor response to immunotherapy, which possibly reflects dysfunctional T-cell states and scarcity of tertiary lymphoid structures[12]. The tumor mutational burden (TMB) is moderate in RCC and does not reliably predict immunotherapy response, distinguishing RCC from other immunogenic cancers[13]. In addition, the metabolic heterogeneity of RCC gives rise to an immunosuppressive TME. Indeed, these features underscore the need for combination strategies that modulate metabolic and immunologic pathways to overcome resistance[14].

Persistent inflammation, a characteristic feature of RCC, significantly influences the onset and progression of disease by shaping the dynamics of immune cells and facilitating immune evasion, angiogenesis, and metastasis by tumor cells[15]. Kidney disease manifests varied impacts on the TME, some of which remain incompletely understood. Cellular metabolism and the fundamental requisites are pivotal for cell proliferation, differentiation, migration, and tissue-specific functions. Metabolic reprogramming, a marker of advanced cancer, affects tumor cell survival and proliferation pathways and modifies the TME[16,17]. Emerging evidence indicates that metabolism regulates immune cell structure, function, and viability. This review discusses our understanding of the interplay between metabolic reprogramming and NET formation in RCC tumorigenesis and metastasis, as well as promising insights into novel therapeutic strategies.

NETs

The immune system is broadly categorized into innate (myeloid) and adaptive (lymphoid) components. Innate immune cells, including macrophages, neutrophils, basophils, eosinophils, mast cells, dendritic cells (DCs), natural killer (NK) cells, and platelets, serve as the first line of defense, neutralizing pathogens and presenting antigens to adaptive immune cells[18]. Adaptive immunity, mediated by T and B lymphocytes, ensures a specific and long-lasting response. The interaction between innate immune cells and the vascular system is crucial for immune surveillance and inflammation with endothelial cells regulating immune cell recruitment and tissue remodeling[19]. Neutrophils, the most abundant leukocyte, are essential for innate immunity by using mechanisms, such as ROS production, phagocytosis, and degranulation[20]. NETs, which are composed of decondensed chromatin and granule proteins, expand the functional repertoire of neutrophils beyond pathogen elimination. The intricate crosstalk between neutrophils, endothelial cells, and the immune system has a pivotal role in inflammation, immune evasion, and tumor progression[18].

Biological characteristics of NETs

NETs are extracellular web-like structures consisting of DNA, histones, and neutrophil granule proteins, including metalloproteinase (MMP), neutrophil elastase (NE), myeloperoxidase (MPO), and cathepsin G (CG)[21]. Initially described as "NETosis," the term was revised in 2018 to "NET formation" by the Nomenclature Committee on Cell Death (NCCD), acknowledging that DNA release can occur independent of neutrophil death[22]. NET formation is triggered by microbial and sterile stimuli, such as phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS), calcium ion flux, and inflammatory cytokines[23].

The process of NET formation involves key proteases, including peptidyl arginine deiminase 4 (PAD4) and NE. PAD4 catalyzes histone citrullination, facilitating chromatin decondensation, while NE disrupts histone-DNA interactions. Gasdermin D forms membrane pores, which allows chromatin extrusion (**Figure 1**) and adds explicit interpretation of

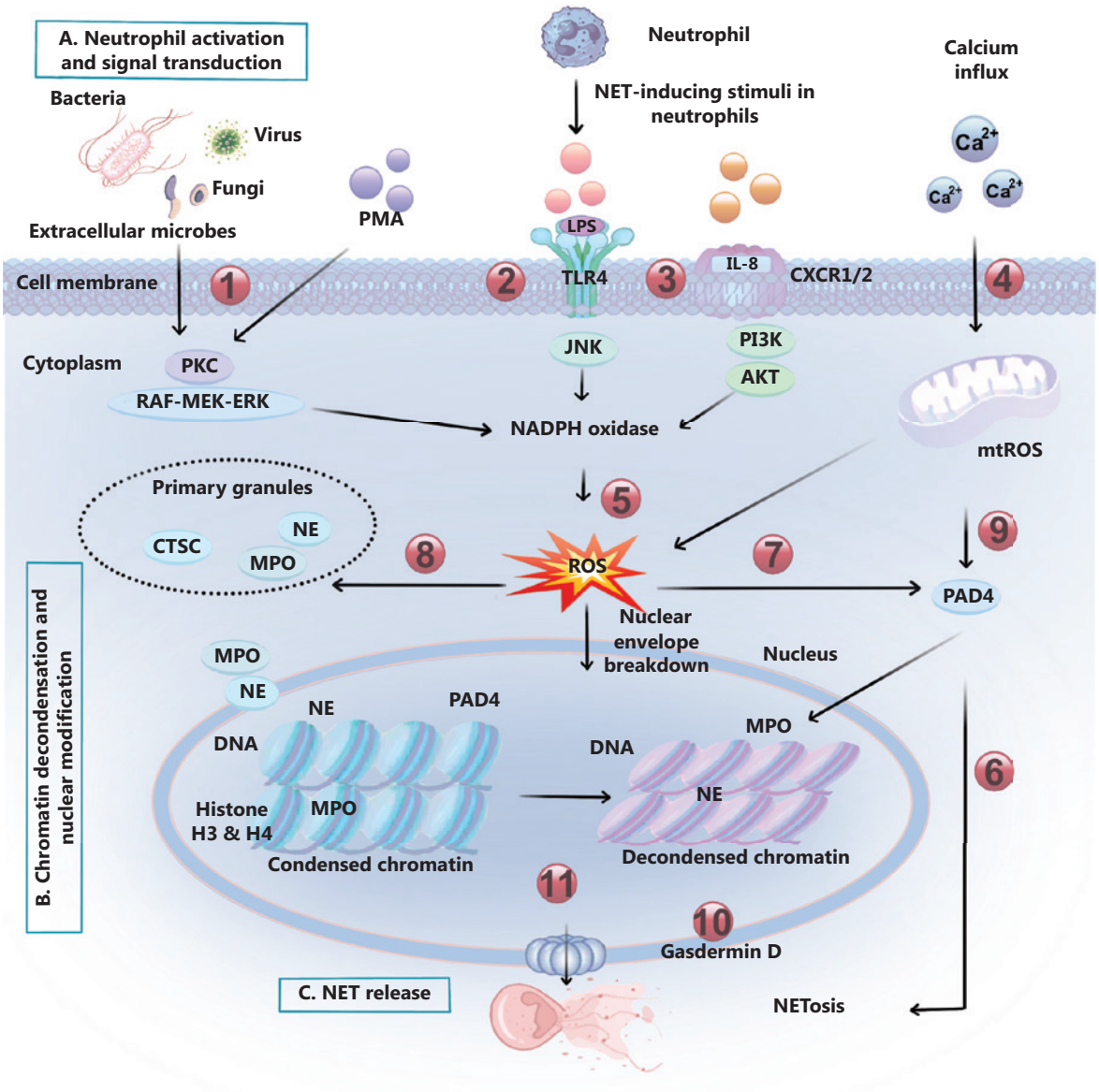

Figure 1 Molecular mechanisms of neutrophil extracellular trap (NET) formation (NETosis). This figure illustrates three sequential stages (A-C) with sub-steps labeled numerically (1–11) to correspond to the signaling events and processes in the diagram. (A) Neutrophil activation and signal transduction (steps 1–6): 1. PMA and extracellular microbe stimulation; PMA and extracellular microbes (bacteria, fungi, and viruses) activate PKC, initiating downstream RAF-MEK-ERK signaling cascades. 2. LPS stimulation; LPS engages TLR4 on the neutrophil membrane, triggering JNK-NADPH oxidase signaling. 3. IL-8 stimulation; IL-8 binds CXCR1/2, leading to PI3K-AKT pathway activation. 4. Calcium influx; chemokine and microbial signals promote calcium entry, which enhances mtROS production. 5. Convergence and amplification of ROS signaling. These pathways (PKC-RAF-MEK-ERK, JNK-NADPH oxidase, and PI3K-AKT) converge on ROS production *via* NADPH oxidase and are further amplified by calcium-driven mtROS production. 6. mtROS-driven PAD4 activation; mtROS promotes NETosis by upregulating PAD4 expression and activity independent of NADPH oxidase. (B) Chromatin decondensation and nuclear modification (steps 7–9): 7. PAD4 activation; ROS and mtROS, along with calcium signaling, upregulate PAD4 expression and enzymatic activity. PAD4 catalyzes the citrullination of histones (H3 and H4), weakening DNA-histone electrostatic interactions and loosening chromatin structure. 8. Granule enzyme translocation; NE and MPO are released from primary azurophilic granules and translocate to the nucleus, where NE and MPO degrade histones and synergize with PAD4-mediated citrullination to further promote chromatin decondensation. 9. mtROS facilitation; mtROS enhanced by calcium influx supports chromatin decondensation through redox-sensitive transcriptional and post-translational mechanisms, reinforcing PAD4 activation and histone modification. (C) NET release (steps 10–11): 10. GSDMD-mediated pore formation; GSDMD forms pores in the nuclear

and plasma membranes, disrupting membrane integrity and enabling the passage of decondensed chromatin. 11. Extracellular chromatin expulsion; the decondensed chromatin, decorated with NE, MPO, PAD4, and MMPs, is released into the extracellular space as NETs. These web-like structures physically entrap pathogens but are also implicated in promoting autoimmunity, thrombosis, and cancer progression by modulating the tumor microenvironment. AKT, protein kinase B; CXCR1/2, c-x-c chemokine receptor types 1 and 2; ERK1/2, extracellular signal-regulated kinase 1/2; GSDMD, Gasdermin D; IL-8, interleukin-8; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MEK, mitogen-activated protein kinase; MMPs, matrix metalloproteinase; MPO, myeloperoxidase; mtROS, Mitochondrial reactive oxygen species; NE, neutrophil elastase; NET, neutrophil extracellular trap; PAD4, peptidyl arginine deiminase 4; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; ROS, reactive oxygen species; TLR4, toll-like receptor 4.

the signaling cascades (e.g., PKC, RAF, ERK1/2, PAD4, and NE)[24]. However, some studies have indicated that NETs can form independent of PAD4-mediated citrullination, underscoring the mechanistic complexity. NET formation is regulated by multiple signaling pathways, including protein kinase C (PKC), extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), AKT, and Src, with additional interplay between inflammasome activation and autophagy[25]. While bacterial infections were initially identified as key inducers of NETs, diverse stimuli, including interleukin-8 (IL-8), PMA, LPS, toxins, ionophores, and activated platelets, can also trigger NET formation. Notably, NET induction varies between *in vitro* and *in vivo* conditions. For example, LPS alone induces NETs *in vitro* but requires platelet interactions *in vivo*[26]. Various techniques are used to detect and analyze NETs, including immunofluorescence, immunohistochemistry, intravital microscopy, DNA-intercalating dyes, and immunoblotting of NET-associated proteins, such as PAD4, NE, MPO, and MMPs. NETs are increasingly recognized as key elements in inflammatory diseases, autoimmune disorders, and malignancies, highlighting the relevance in cancer progression and therapeutic targeting[27].

Metabolic requirements for NET formation

NETs are extracellular chromatin networks decorated with antimicrobial proteins. such as MPO, NE, and histones. The process of NET formation, termed NETosis, occurs *via* cell death- and non-cell death-dependent mechanisms[28]. Recent studies have described acute NETosis as a novel, non-lytic form of NET release. The metabolic requirements for NET formation highlight glycolysis as a key pathway. PMA-induced NETosis in human neutrophils requires exogenous glucose and glutamine with inhibition by 2-2-deoxy-D-glucose (2-DG) blocking NET release[29,30]. While ATP synthase inhibition by oligomycin only partially reduces NET formation, the pentose phosphate pathway has a pivotal role by generating NADPH, which fuels NADPH oxidase and ROS production. ROS further upregulates PAD4, catalyzing histone citrullination, chromatin decondensation, and NET extrusion[31,32].

Mitochondrial complex I activity also contributes to NETosis. Inhibition of glycolysis by 2-DG reduces NET release in human and mouse neutrophils in GM-CSF + C5a-induced NETosis[33]. Similarly, platelet-activating factor (PAF)-induced NETosis is suppressed by glycolysis inhibition in bovine neutrophils, as summarized in **Figure 2**. Recent findings suggest that NET formation and function may vary across RCC subtypes due to differential genetic and metabolic profiles. Clear cell renal cell carcinoma (ccRCC), the most prevalent subtype of RCC, is typically characterized by von Hippel-Lindau (*VHL*) gene mutations leading to hypoxia-inducible factor (HIF) stabilization and subsequent VEGF-A overexpression, which promotes angiogenesis and neutrophil infiltration[34,35]. These features potentially enhance NET formation through increased IL-8 and G-CSF signaling within the TME. Conversely, papillary and chromophobe RCC subtypes often lack *VHL* inactivation and demonstrate less immune infiltration, potentially resulting in a reduced propensity for NET generation[35,36]. Metabolic signatures differ among subtypes, with ccRCC showing greater glycolytic flux and lipid accumulation, conditions that are favorable for NETosis *via* ROS-mediated pathways. These distinctions underscore the need for subtype-specific strategies when targeting NETs in RCC[17,37]. Furthermore, NETosis is linked to purinergic signaling because pharmacologic inhibition of P2X1 receptors effectively blocks NET formation[38,39]. Bovine neutrophils infected with *Besnoitia biscotti* tachyzoites exhibit increased glucose consumption but the NET release remains unaffected by glycolysis inhibition, instead relying on mitochondrial ATP and P2X1 receptor signaling. Non-esterified fatty acids (NEFAs) also trigger NETosis with inhibition of P2X1 receptors and β-oxidation reducing NET formation[40,41].

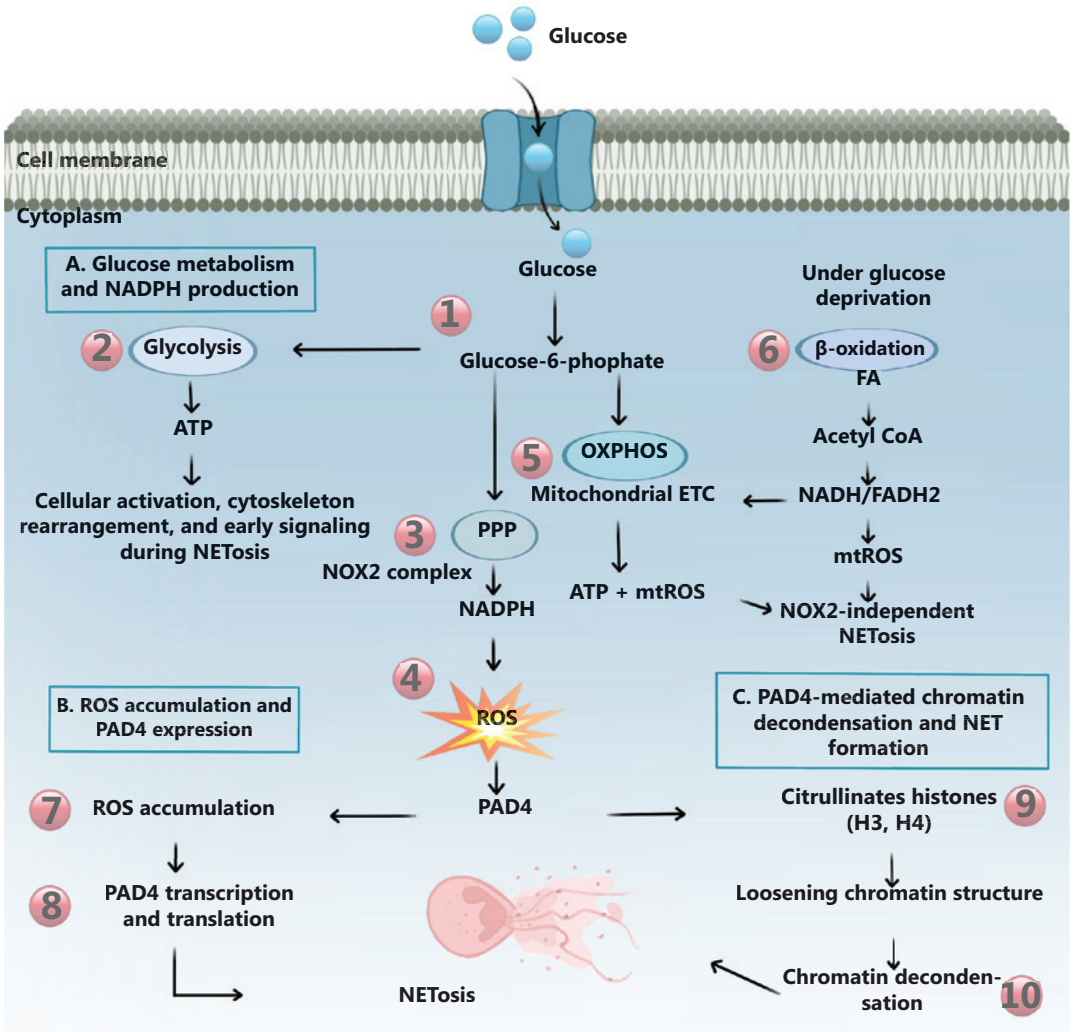

Figure 2 Glucose metabolism-mediated ROS generation and PAD4 transcriptional upregulation in NETosis. This schematic illustrates three interconnected stages (A-C) depicting the metabolic and redox-regulated mechanisms driving NET formation. (A) Glucose metabolism and NADPH production (1–6): 1. Glucose uptake; glucose is transported into the neutrophil *via* the GLUT1 transporter, enabling metabolic fuel availability for downstream pathways. 2. Glycolysis; Following cellular uptake, glucose undergoes glycolysis, producing ATP and key metabolic intermediates. the glycolysis-derived ATP fuels cellular activation, cytoskeletal remodeling, and early signaling processes that are essential for initiating NETosis. 3. PPP; A portion of glucose flux is diverted into the PPP, which serves as the principal source of NADPH required for redox balance and ROS generation. 4. NADPH production and ROS generation; NADPH provides reducing equivalents that drive the production of cytosolic ROS, which is essential for classical NETosis. 5. Mitochondrial contributions; Mitochondrial OXPHOS produces ATP and mtROS, supporting NOX2-independent NET formation, particularly under glucose-restricted or inflammatory conditions. 6. Fatty acid β-oxidation; under metabolic stress conditions, β-oxidation of fatty acids generates acetyl-CoA, NADH, and FADH2, which fuel the ETC, leading to increased mtROS production that sustains redox signaling required for NETosis. (B) ROS accumulation and PAD4 expression (7, 8): 7. ROS accumulation; accumulated ROS derived from both NADPH and mitochondria act as upstream signals that activate redox-sensitive transcription factors. 8. PAD4 gene transcription; these transcription factors upregulate PAD4 gene expression, increasing PAD4 protein levels and enzymatic activity within neutrophils. (C) PAD4-mediated chromatin decondensation and NET formation (9, 10): 9. Histone citrullination; PAD4 catalyzes citrullination of histones H3 and H4, reducing electrostatic DNA-histone interactions and loosening chromatin structure. 10. Chromatin decondensation; NE and MPO translocate to the nucleus, further promoting histone degradation and chromatin decondensation. The ruptured nuclear and plasma membranes release decondensed chromatin into the extracellular space as NETs. The arrows in the schematic indicate the sequential flow of metabolic and signaling events culminating in NET formation. ATP, adenosine triphosphate; GLUT1, glucose transporter 1; MPO, myeloperoxidase; mtROS, mitochondrial reactive oxygen species; NE, neutrophil elastase; NOX2, NADPH2 oxidase; OXPHOS, oxidative phosphorylation; PAD4, peptidyl arginine deiminase 4; PPP, pentose phosphate pathway; ROS, reactive oxygen species.

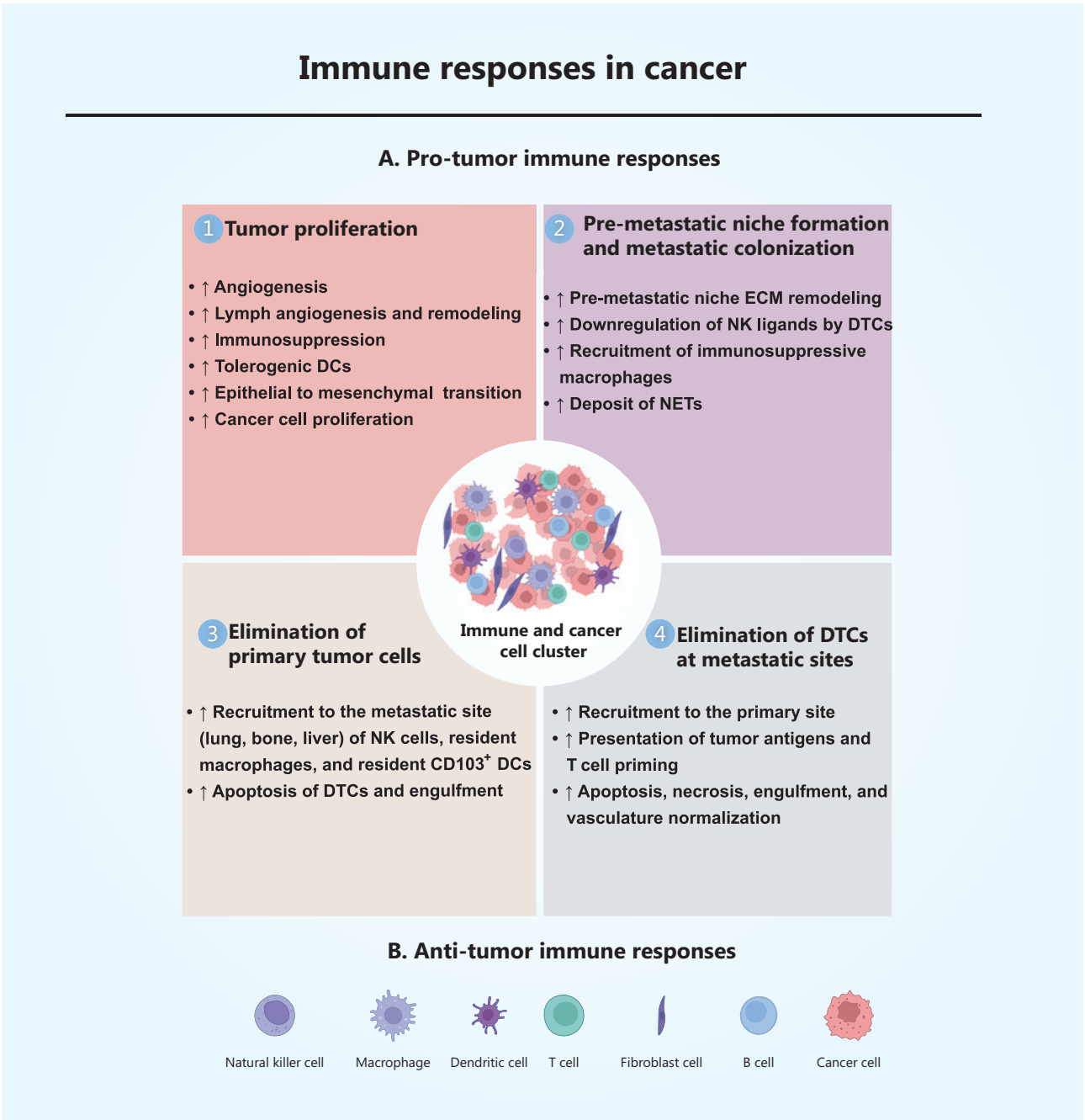

Figure 3 Dual roles of innate immune cells in tumor progression and metastasis. This schematic illustrates the opposing functions of innate immune cells in shaping cancer progression, immune surveillance, and metastatic colonization. The central cluster represents the heterogeneous mix of immune and cancer cells within the tumor microenvironment, highlighting dynamic interactions that shift the balance between pro- and anti-tumor immune responses and ultimately determine tumor fate. (A) Pro-tumor immune responses (1, 2): innate immune cells, including TAMs, TANs, and tolerogenic or immature DCs, contribute significantly to tumor progression through multiple coordinated mechanisms. 1. Tumor proliferation; these cells promote angiogenesis, lymphangiogenesis and remodeling, immunosuppression, the expansion of tolerogenic DC populations, EMIT, and increased cancer cell proliferation. 2. Pre-metastatic niche formation and metastatic colonization; this stage involves ECM remodeling, downregulation of NK cell ligands by DTCs, recruitment of immunosuppressive macrophages, and deposition of NETs. These coordinated processes collectively establish a permissive microenvironment that facilitates metastatic seeding, immune evasion, and distant tumor colonization. (B) Anti-tumor immune responses (3, 4): activated innate immune cells, including NK cells, cytotoxic

macrophages, and mature CD103$^+$ DCs, have play critical roles in controlling tumor progression *via* two key mechanisms. 3. Elimination of primary tumor cells; at the primary tumor site, these cells enhance immune surveillance through increased recruitment, presentation of tumor antigens, T cell priming, and the induction of apoptosis, necrosis, engulfment, and vascular normalization. 4. Elimination of DTCs at metastatic sites; in metastatic sites, such as the liver, lung, and bone, resident NK cells, macrophages, and DCs are similarly recruited to target DTCs, mediating apoptosis and engulfment. The effectiveness of these responses depends on the polarization state of the innate immune cells, which can suppress or facilitate metastatic colonization, underscoring the dynamic balance between anti-tumor immunity and immune evasion in the tumor microenvironment. DC, dendritic cell; DTC, disseminated tumor cell; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; NET, neutrophil extracellular trap; NK, natural killer cell; TAM, tumor-associated macrophage; TAN, tumor-associated neutrophil.

Dysregulation of NETs in disease pathogenesis

NETs are decorated with histones and granular proteins, including CG, NE, proteinase 3 (PR3), MMP-9, lactotransferrin, LL-37, calprotectin, and pentraxin 3[42]. These components enhance NET antimicrobial activity but also contribute to pathologic conditions. Excessive NET formation is implicated in thrombosis, atherosclerosis, rheumatoid arthritis, cystic fibrosis, asthma, chronic inflammatory diseases, ischemia-reperfusion injury, acute respiratory distress syndrome (ARDS), and cancer[43-45].

NETs promote cancer progression within the TME by influencing angiogenesis, cancer cell motility, invasion, metastasis, and immune evasion. NET-derived proteases and DNA scaffolds regulate these processes, modulating immune surveillance and stromal cell interactions. Increasing evidence links NET production with tumor development, making NET-targeted therapies an emerging avenue for cancer treatment. Understanding NET regulation and the role of NETs in cancer and inflammatory disorders may facilitate novel therapeutic strategies[45,46].

Immunotherapy and NETs in cancer

Cancer comprises a heterogeneous group of diseases characterized by uncontrolled cell proliferation. Standard treatments for cancer, including surgery, radiotherapy, and chemotherapy, continue to face challenges, such as limited efficacy, adverse effects, and resistance in advanced-stage cancers[47,48]. The immune system has a vital role in suppressing and promoting tumor progression. Immune surveillance prevents malignancy in healthy individuals, but in cancer patients immune evasion mechanisms enable tumor growth. Tumor-associated immune cells contribute to disease progression by facilitating angiogenesis, metastasis, and therapy resistance through cytokine and growth factor secretion[49,50]. Tumor-associated macrophages (TAMs) are key cells in tumor progression from early neoplastic transformation to metastasis (**Figure 3**) with expanded innate immune cell roles in tumorigenesis.

NETs contribute to tumorigenesis by promoting cancer cell proliferation, differentiation, and metastasis. NE degrades the extracellular matrix (ECM) and activates the PI3K pathway, enhancing cancer cell migration[51]. MMPs drive tumor expansion through ECM remodeling, while NET-associated proteases stimulate integrin signaling, potentially reactivating dormant tumor cells[52].

Additionally, NETs shield cancer cells from immune clearance by cytotoxic T lymphocytes (CTLs) and NK cells. High intertumoral NET levels and elevated serum MPO-DNA correlate with metastasis, supporting cancer cell survival under stress[53]. Although NETs are predominantly associated with pro-tumorigenic processes, emerging evidence suggests that NETs may also exert antitumor effects under specific conditions. NETs have been shown to entrap and damage circulating tumor cells (CTCs), thereby potentially limiting metastatic dissemination[54]. In addition, NETs enhance the recruitment and activation of cytotoxic immune cells, including NK cells and CTLs, contributing to improved tumor surveillance. It has also been suggested that NETs modulate the tumor-associated microbiome, suppressing pro-carcinogenic microbial populations[55]. These findings underscore the dual role of NETs in cancer biology and suggest that the functional consequences may vary depending on tumor type, microenvironmental context, and systemic inflammatory status.

Cancer-associated platelet activation enhances metastatic potential by protecting tumor cells, promoting the epithelial-mesenchymal transition (EMT) and releasing pro-tumor growth factors. NETs contribute to thrombosis, including arterial, venous, and cancer-associated thrombosis, suggesting that NET-targeted therapies may mitigate thrombotic complications and tumor progression[56,57]. NETs sustain a pro-tumor milieu within the tumor immune microenvironment (TIME)

by stimulating cancer-associated fibroblast (CAF) conversion *via* NF-κB signaling, fostering fibroblast growth factor (FGF) production and creating a tumor-supportive niche. While NETs primarily facilitate tumor progression, some evidence suggests NETs may possess anti-tumor properties by targeting cancer cells and modulating immune responses[58,59]. NETs may suppress cancer-associated microbiota in colorectal cancer, which limits tumor spread. The mechanisms regulating NET formation in cancer involve TLR4 and HMGB1-dependent pathways in lung cancer and MPO NET-ANCA signaling in chronic inflammation. Understanding the dual role of NETs is essential for developing targeted therapies that inhibit the pro-tumorigenic effects or harness the potential for cancer suppression[59,60].

NETs in RCC

RCC exhibits high resistance to chemotherapy and radiotherapy with a poor prognosis in advanced cases. While targeted therapies offer some benefits, the efficacy varies by subtype, which necessitates alternative approaches, such as immunotherapy[61]. Increasing evidence suggests that immune components, including T cells, NK cells, DCs, and neutrophils, significantly influence RCC progression. Tumor-associated neutrophils (TANs) are key myeloid infiltrates in RCC, the presence of which correlates with poor clinical outcomes. Both tumor-infiltrating neutrophils (TINs) and peripheral blood neutrophils have been implicated in RCC progression, although the exact interplay has not been established[62,63].

Role of NETs in RCC progression

NETs composed of DNA scaffolds decorated with neutrophil-derived proteins contribute to cancer progression by promoting tumor cell invasion, proliferation, and metastasis. NETs interact with CTCs in RCC, facilitating immune evasion and metastatic dissemination[61,64]. NETs also have a role in thrombosis, a frequent occurrence in RCC, suggesting a mechanistic link between inflammation, coagulation, and tumor survival. Gene expression studies across multiple cancers, including RCC, indicate that NET-associated signatures correlate with poor prognosis[62].

NET-associated proteins in RCC pathophysiology

Key NET-associated proteins, such as MPO, NE, and MMP-9 are implicated in RCC progression[63]. NE enhances tumor proliferation and migration, while MMP-9 contributes to metastasis. In addition, IL-8 promotes NET formation and angiogenesis, sustaining a pro-TME, as summarized in **Figure 4**[65]. Granulocyte colony-stimulating factor (G-CSF) primes neutrophils for NETosis, further accelerating tumor progression. Interestingly, PAD4 deficiency reduces tumor burden only in G-CSF-producing tumors, underscoring the complexity of NET-driven oncogenesis. Further investigation into NET-targeted interventions may provide novel therapeutic strategies for RCC management[66-68].

Interactions between NETs and metabolic reprogramming in RCC

The metabolic demands of RCC cells lead to profound alterations in the TME, which influences immune responses. The disorganized vasculature within the TME results in nutrient scarcity and hypoxia, intensifying competition between cancer and immune cells[11,69]. Cancer cells prioritize glycolysis by upregulating rate-limiting enzymes, limiting glucose availability to antitumor immune cells, and impairing effector functions. In addition, the accumulation of immunosuppressive metabolites, such as adenosine, kynurenine, ROS, and lactate, further suppress immune responses[15]. These metabolic adaptations support tumor survival and immune evasion, highlighting the necessity of targeting metabolic crosstalk for improved immunotherapy efficacy and explaining the immunometabolic alterations in the TME, which are summarized in **Figure 5**. Malignant RCC cells undergo metabolic reprogramming, utilizing extracellular and endocytic pathways to sustain proliferation[70]. While normal cells metabolize glucose *via* oxidative phosphorylation, RCC cells favor aerobic glycolysis, converting pyruvate to lactate *via* lactate dehydrogenase (LDHA), even under normoxic conditions. This metabolic shift supports biosynthetic needs but also fosters an immunosuppressive TME[64,71].

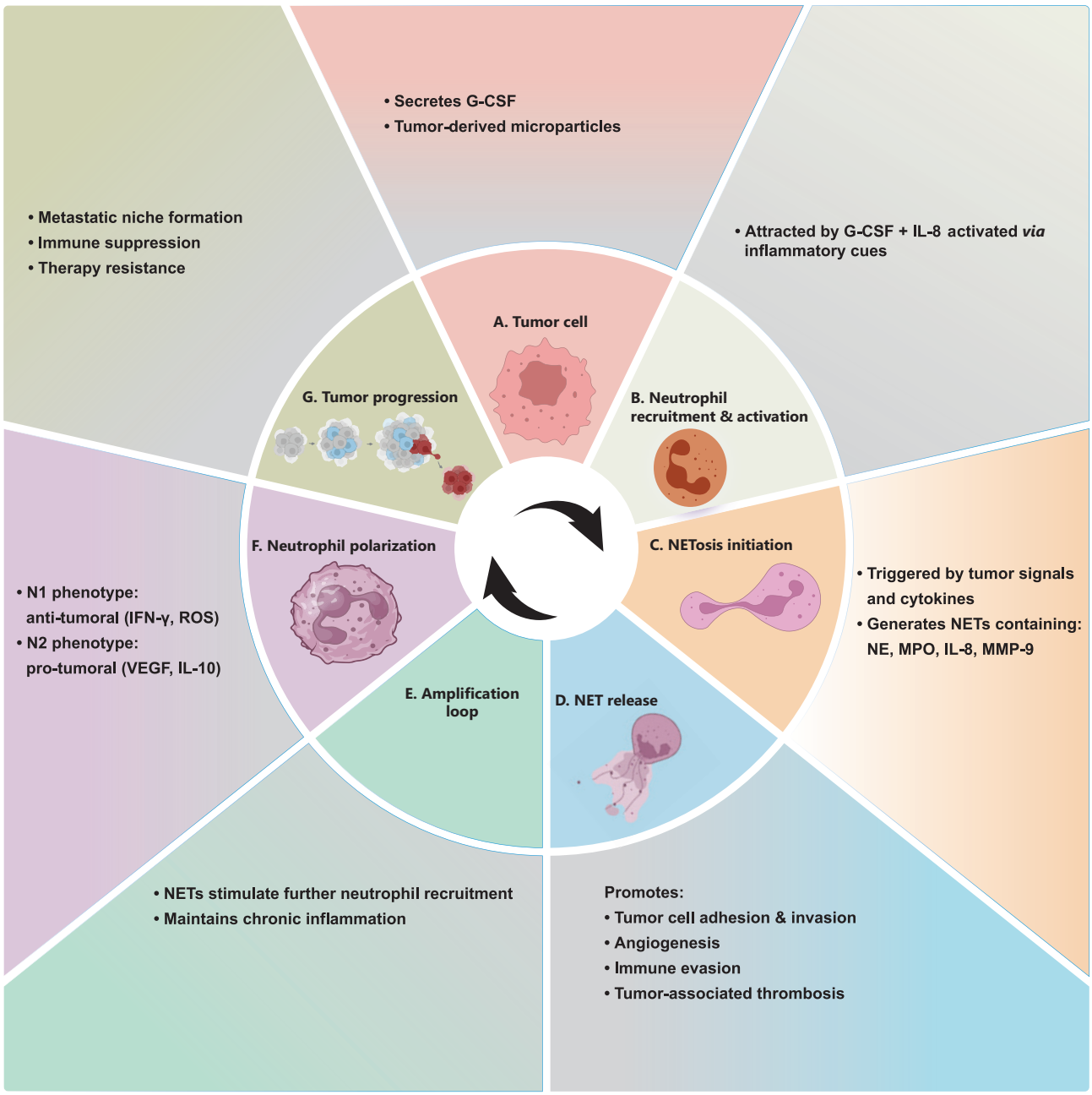

Figure 4 Reciprocal interaction between tumor cells, neutrophils, and neutrophil extracellular traps (NETs) in cancer progression. This schematic illustrates the sequential and reciprocal crosstalk between tumor cells and neutrophils, emphasizing how NETs promote cancer progression through multiple interconnected processes. (A) Tumor cell: tumor cells release G-CSF and microparticles, which initiate neutrophil recruitment and activation within the tumor microenvironment. (B) Neutrophil recruitment & activation: activated neutrophils respond to tumor-derived factors and inflammatory cytokines, including IL-8, enhancing their migration, priming, and readiness to undergo NETosis. (C) NETosis initiation: stimulated neutrophils extrude chromatin fibers decorated with NE, MPO, IL-8, and MMP-9, forming structurally complex and functionally active NETs. (D) NET release: these extracellular NET structures facilitate tumor progression by enhancing cancer cell adhesion and invasion, promoting angiogenesis, suppressing local immune responses, and contributing to tumor-associated thrombosis. (E) Amplification loop: NETs reinforce neutrophil recruitment and activation, establishing a self-perpetuating pro-inflammatory feedback loop that maintains a tumor-promoting microenvironment. (F) Neutrophil polarization: within the microenvironment, neutrophils can polarize toward either an antitumor (N1) or protumor (N2) phenotype depending on local factors, further shaping disease progression. (G) Tumor progression: collectively, these interconnected processes support metastatic niche formation, enable immune evasion, and contribute to therapy

resistance, underscoring the complex role of neutrophil-tumor interactions in cancer biology. G-CSF, granulocyte colony-stimulating factor; IL-8, interleukin-8; MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; NE, neutrophil elastase; NET, neutrophil extracellular trap.

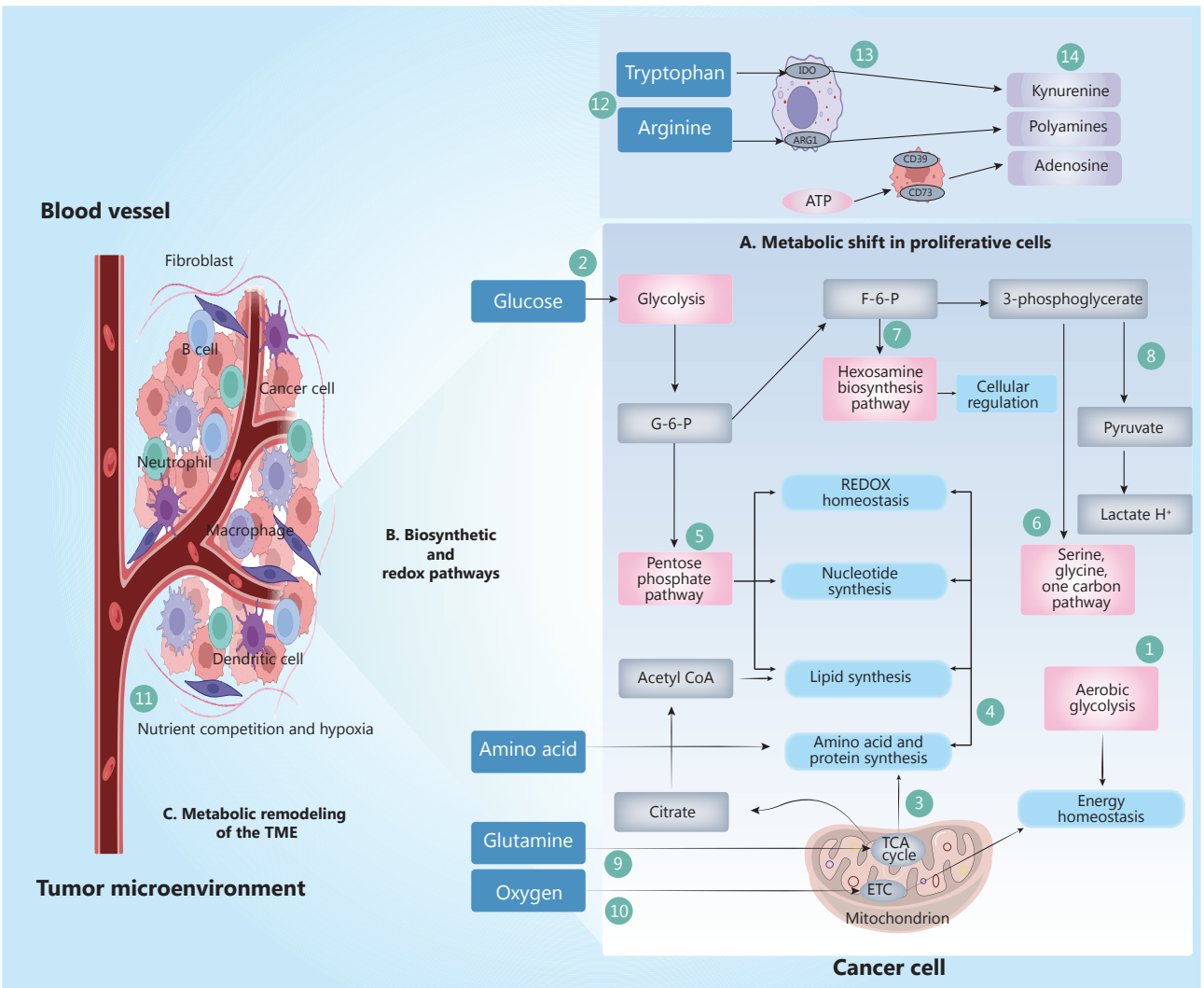

Figure 5 Metabolic reprogramming of cancer cells and the immunosuppressive modulation of the tumor microenvironment (TME). This schematic illustrates the stepwise metabolic adaptations in proliferative cancer cells and the consequences for immune suppression within the surrounding tumor microenvironment. (A) Metabolic shift in proliferative cells (1–4): 1. Aerobic glycolysis (Warburg effect); rapidly dividing cancer cells shift from oxidative phosphorylation to aerobic glycolysis, despite its lower ATP yield, to sustain rapid proliferation. 2. Glucose uptake and utilization; increased glucose uptake fuels this pathway, providing a continuous source of carbon. 3. Mitochondrial oxidative metabolism in quiescent cells; in contrast, non-proliferating, differentiated cells primarily rely on mitochondrial OXPHOS *via* the TCA cycle and ETC for efficient ATP production. 4. Redirection of intermediates; glycolytic intermediates are rerouted into anabolic pathways, supplying precursors for nucleotide, amino acid, and lipid biosynthesis as well as regulatory networks that sustain rapid cell growth and division. (B) Biosynthetic and redox pathways (5–10): 5. Pentose phosphate pathway; glycolytic intermediates feed into the PPP, generating NADPH for reductive biosynthesis and maintaining redox balance. 6. Serine-glycine-one-carbon metabolism; glycolytic intermediate 3-phosphoglycerate is diverted into this pathway, supporting nucleotide synthesis and methylation reactions essential for sustained cellular proliferation. 7. Hexosamine synthesis; F-6-P from glycolysis is utilized in this pathway to produce substrates required for protein and lipid glycosylation, as well as other cellular regulatory processes essential for tumor growth. 8. Conversion to pyruvate and lactate production: in parallel, 3-phosphoglycerate

also continues through glycolysis to form pyruvate, which is converted to lactate and H^+. This lactate production contributes to acidification of the tumor microenvironment and supports immune evasion. 9. Glutamine metabolism; glutamine-derived α-ketoglutarate replenishes TCA cycle intermediates (anaplerosis) and supports anabolic processes, such as amino acid and nucleotide synthesis. 10. Oxygen consumption in mitochondria; oxygen from the surrounding microenvironment is taken up by cancer cells and utilized as the terminal electron acceptor in mitochondrial OXPHOS, supporting ATP production while contributing to local hypoxia in the tumor microenvironment. (C) Metabolic remodeling of the TME (11–14): 11. Nutrient competition and hypoxia; High metabolic demand creates nutrient-depleted and hypoxic conditions that impair immune cell function. 12. Accumulation of metabolic byproducts; metabolites, such as tryptophan and arginine, accumulate in the TME, leading to acidification and generating immunosuppressive signals that inhibit effector immune cell function. 13. Expression of immunomodulatory ectoenzymes; tumor and stromal cells express enzymes, such as IDO, ARG1, and CD39/73, which degrade tryptophan, arginine, and ATP, respectively. 14. Production of immunosuppressive metabolites; resulting metabolites, like kynurenine and adenosine, inhibit T cell and natural killer cell activity, promoting immune evasion. ARG1, arginase 1; CD39/73, ecto-5′-nucleotidase; ETC, electron transport chain; IDO, indoleamine 2,3-dioxygenase; NAD, nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PPP, pentose phosphate pathway; TCA, tricarboxylic acid cycle; TME, tumor microenvironment.

Impact of NETs on the TME and immune modulation

The TME comprises a dynamic network of ECM, stromal cells, and immune infiltrates, collectively shaping tumor progression[58]. Immune cells, including DCs, NK cells, neutrophils, and macrophages, are critical in regulating tumor immunity. Neutrophils recruited into the TME differentiate into TANs, displaying an antitumor (N1) or tumor-promoting (N2) phenotype[72]. TANs influence angiogenesis, invasion, and metastasis, serving as predictive markers for RCC prognosis. Neutrophils, a major component of the immune infiltrate in RCC, rely primarily on glycolysis for energy. The persistence of neutrophils within tumors is supported by tissue-derived survival signals and chemokines, such as CXCL2 and CXCL8, which attract neutrophils and sustain neutrophil function[52,73]. Neutrophils contribute to tumor progression within the TME by secreting oncostatin M (OSM) and promoting endothelial disruption, which enhances metastasis. Elevated NET release has been observed in various malignancies, including hepatocellular carcinoma (HCC) and RCC, facilitating cancer cell migration and immune evasion[52].

NET-associated factors and RCC metabolism

NETs have a pivotal role in modulating metabolic pathways within RCC. NET-derived proteins, such as NE and MMPs contribute to ECM remodeling, enhancing tumor invasion[74,75]. The involvement of NETs in metabolic reprogramming remains underexplored but emerging evidence suggests that the presence or NETs within the TME may alter immune cell metabolism, impairing antitumor responses. Recent studies have revealed that NET-associated enzymes, such as NE and MPO, can indirectly influence glycolytic enzymes and mitochondrial respiration in tumor cells, thereby contributing to metabolic reprogramming[28,76]. Additionally, NET-derived ROS may stabilize HIF-1α and activate the mTOR signaling pathway, facilitating aerobic glycolysis and tumor proliferation. With respect to immune checkpoints, NETs have been shown to upregulate PD-L1 expression *via* Toll-like receptor 4 (TLR4)-NF-kB signaling in tumor-associated immune cells, linking innate immune responses to adaptive immune evasion[77,78]. These mechanisms underscore the multifaceted influence of NETs in shaping the metabolic and immunologic landscape of RCC. Further investigation into NET-mediated metabolic modulation could uncover novel therapeutic targets for RCC[79,80].

Novel immune-based therapies for RCC

RCC cancer-immunity cycle

The cancer-immunity cycle, first described by Chen and Mellman in 2013, outlines key biological stages essential for an effective immune response against tumors. RCC exhibits responsiveness to immunotherapy despite lacking classical immune-reactive features that exist in melanoma or non-small cell lung cancer[81,82]. The RCC-specific cancer-immunity cycle highlights the unique roles of immune components and immunotherapeutic strategies, which are summarized in

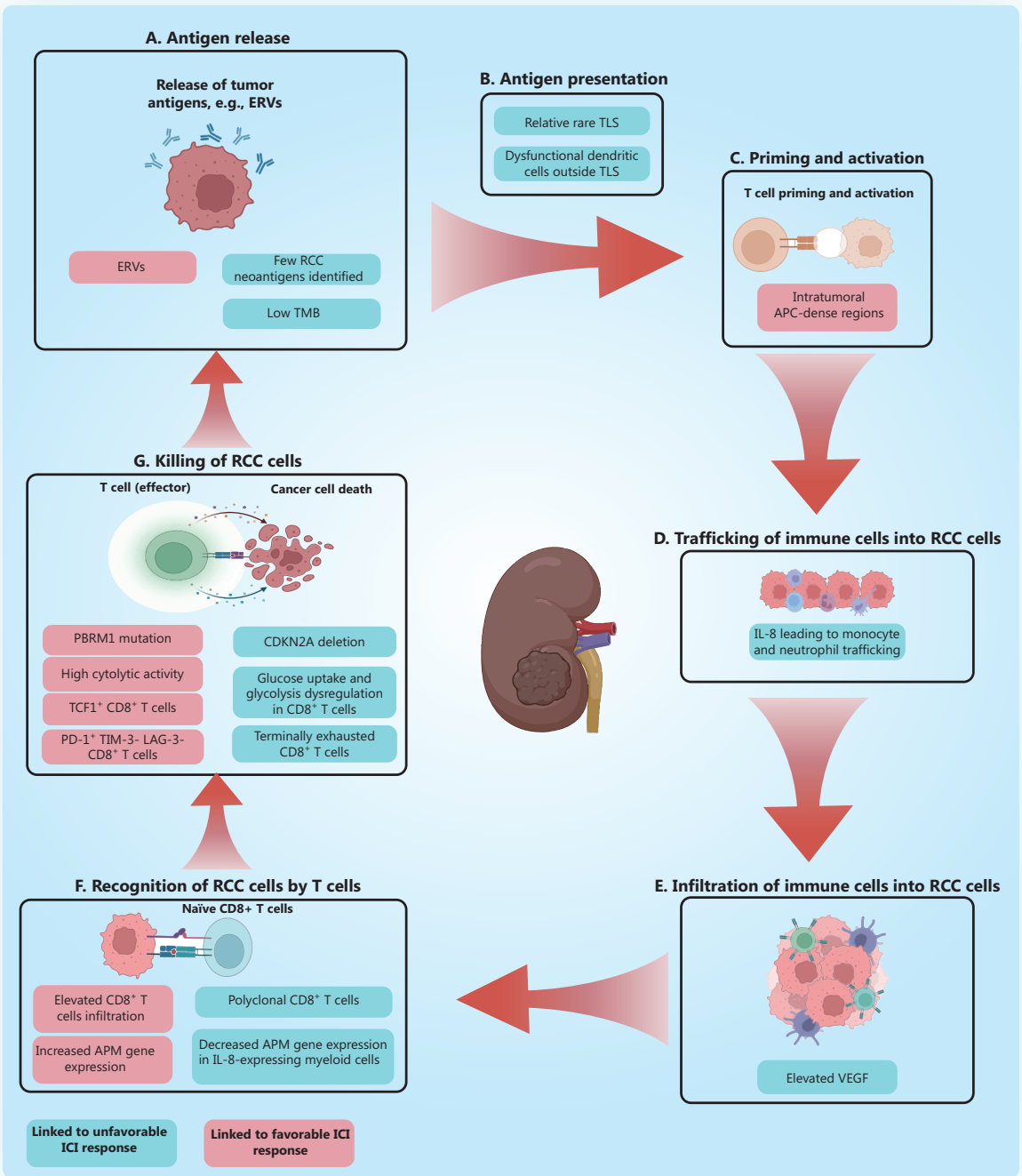

Figure 6 Immune landscape and checkpoint regulation in renal cell carcinoma (RCC). This schematic illustrates the RCC-specific adaptation of the cancer-immunity cycle, emphasizing immunologic features characteristic of ccRCC that influence immune surveillance and response to ICIs. These features include neoantigen release, antigen presentation, T cell priming, tumor infiltration, immune evasion, and cytotoxic resistance within a metabolically constrained TME. (A) Antigen release: tumor cell death in RCC leads to the release of cancer-specific antigens,

including ERV-derived peptides. However, the number of identifiable neoantigens is limited due to the typically low TMB in RCCs, which constrains antigenic diversity and may impact immunogenicity. (B) Antigen presentation: released antigens are processed and presented by APCs, particularly DCs. While intratumoral APC-rich zones support presentation, the relative scarcity of TLSs and the presence of dysfunctional DCs outside TLSs may compromise efficient antigen presentation. (C) Priming and activation: in lymphoid organs or APC-dense tumor regions, $CD8^+$ T cells are activated upon encountering antigen-loaded DCs, triggering clonal expansion and effector differentiation. The antigen repertoire and TCR diversity influence the strength and breadth of this response. (D) Trafficking of immune cells into RCC cells: effector T cells migrate to the tumor guided by chemokine gradients and adhesion signals. IL-8 plays a prominent role in RCC by driving the recruitment of monocytes and neutrophils, which can skew the immune landscape and alter trafficking patterns. (E) Infiltration of immune cells into RCC cells: infiltration of T cells into tumor tissues is modulated by both vascular factors and immunosuppressive signaling. Elevated VEGF levels in ccRCC may impair endothelial function, hinder T cell entry, and promote immunosuppressive stroma. (F) Recognition of RCC cells by T cells: RCC tumors often show high $CD8^+$ T cell infiltration and upregulation of APM genes, enhancing immunogenic recognition. However, polyclonal $CD8^+$ T cells and IL-8-producing myeloid cells with reduced APM expression can impair T cell targeting accuracy and functional responses. (G) Killing of RCC cells: effective cytolytic activity is associated with molecular and phenotypic features, including PBRM1 mutations, $TCF1^+$ $CD8^+$ T cells, and $PD\text{-}1^+$ $TIM\text{-}3^-$ $LAG\text{-}3^-$ effector phenotypes. Conversely, mechanisms of resistance include CDKN2A deletion, dysregulation of glucose metabolism and glycolysis in $CD8^+$ T cells, and terminal T cell exhaustion, all of which compromise tumor cell killing. APC, antigen-presenting cell; APM, antigen presentation machinery; ccRCC, clear cell renal cell carcinoma; CDKN2A, cyclin-dependent kinase inhibitor 2A; DC, dendritic cell; ERV, endogenous retrovirus; ICI, immune checkpoint inhibitor; IL-8, interleukin-8; LAG-3, lymphocyte activation gene 3; MHC1, major histocompatibility complex class 1; PBR1, polybromo 1; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; RCC, renal cell carcinoma; TCR, T cell receptor; TCF1, T cell factor 1; TME, tumor microenvironment; TMB, tumor mutational burden; TLS, tertiary lymphoid structure; VEGF, vascular endothelial growth factor.

Figure 6. Targeting NETs and associated metabolic pathways has the potential to modulate multiple phases of the RCC cancer immunity cycle[11]. For example, DNase 1-mediated NET degradation disrupts physical barriers that prevent T-cell infiltration and reverts the EMT, thereby improving immune cell recognition of tumor cells. Inhibitors of PAD4 or NE can diminish immunosuppressive NET scaffolds and reduce pro-inflammatory cytokine release, ultimately enhancing antigen presentation and T-cell priming[83]. Moreover, metabolic interventions, such as LDHA inhibition or glycolysis blockade, reduce lactate accumulation, which restores T-cell effector function and improves tumor cell killing. These strategies exemplify how the integration of NETs and metabolism can recalibrate immune dynamics and overcome immunotherapy resistance in RCC[84]. Neoantigens, arising from somatic mutations, are pivotal in anti-tumor immunity. However, RCCs exhibit moderate the TMB and the TMB does not predict clinical outcomes in RCC, unlike other ICI-responsive tumors. Although RCCs frequently harbor frameshift indels, which theoretically enhance immunogenicity, RCCs do not correlate with immune checkpoint inhibitor (ICI) responsiveness. Other tumor-associated antigens, such as cancer-testis antigens and endogenous retroviruses, show inconsistent roles in RCC immunity[85-87].

ccRCC is characterized by high immune infiltration and angiogenesis and is largely driven by *VHL* mutations and VEGF-A overexpression[88]. This finding contradicts preclinical findings in which VEGF-A upregulation has been shown to suppress immune cell infiltration. RCC subtypes differ in immune infiltration, angiogenic profiles, and molecular signatures, which affect prognosis and response to therapy. Notably, tumors with high VEGF expression exhibit decreased immune infiltration and improved outcomes, whereas tumors with enhanced antigen presentation machinery activity tend to have worse prognoses[89]. Paradoxically, $CD8^+$ T-cell infiltration in RCC is linked to poor survival, which is likely due to the scarcity of tertiary lymphoid structures and metabolic dysregulation impairing T-cell function. Moreover, genomic alterations, such as 9p21.3 loss (CDKN2A/CDKN2B) and absence of PBRM1 mutations, influence immune interactions and clinical outcomes[90-92].

Targeting NETs in RCC

NETs contribute to RCC progression by modulating metabolic reprogramming, immune evasion, and metastasis[62]. NET-targeted therapies offer the potential to reduce metastasis and enhance immunotherapy efficacy. However, neutrophil depletion is limited by infection risks, making NET disruption a more viable approach, as summarized in **Figure 7**[61].

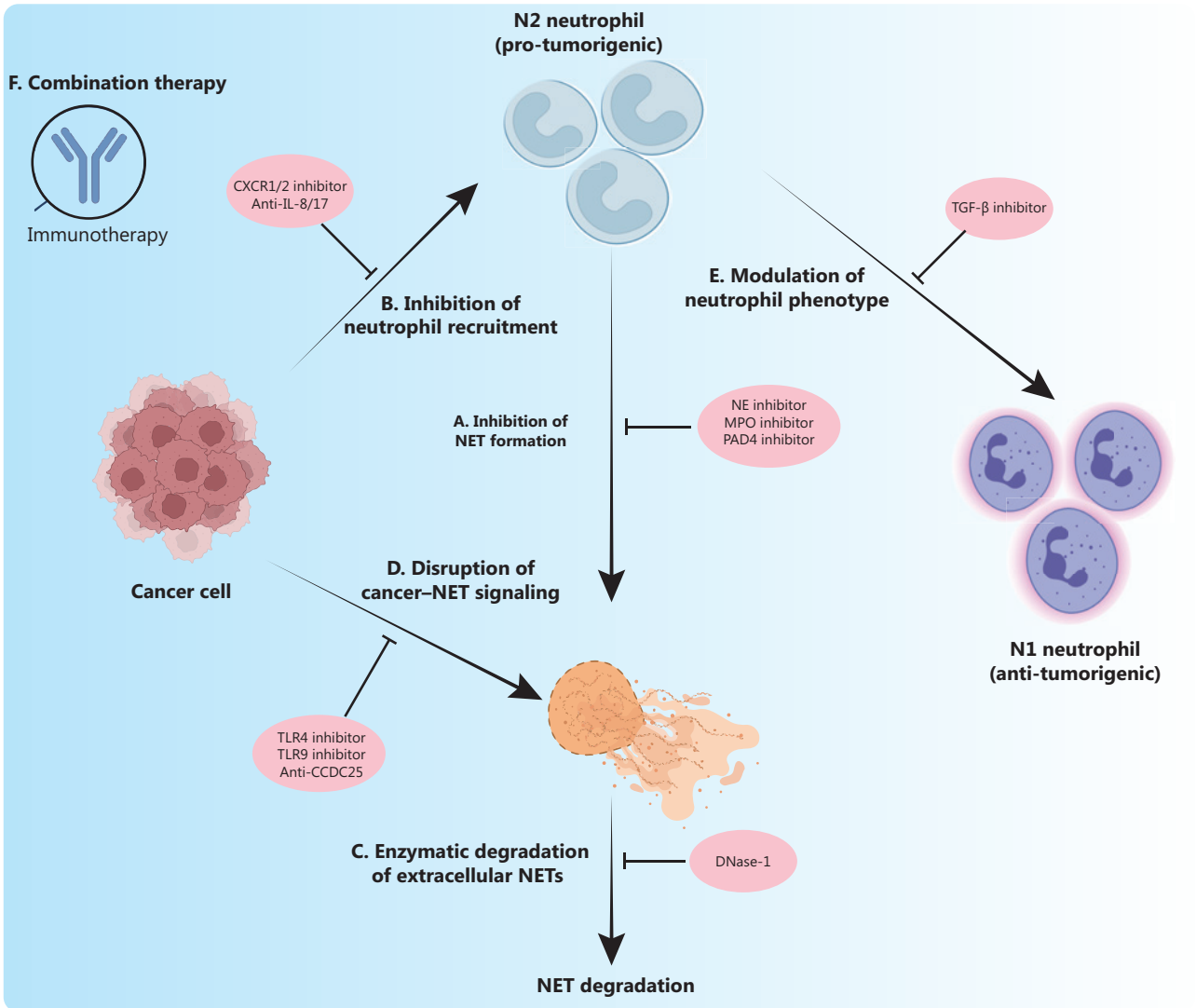

Figure 7 Therapeutic strategies targeting neutrophil extracellular traps (NETs) in renal cell carcinoma (RCC). This schematic summarizes emerging approaches designed to disrupt NET-mediated tumor progression in RCC, highlighting their potential to synergize with immunotherapeutic interventions to enhance overall antitumor efficacy. (A) Inhibition of NET formation: pharmacologic blockade of key enzymes, including NE, MPO, and PAD4, prevents histone citrullination and chromatin decondensation, thereby reducing NET release from activated neutrophils. (B) Inhibition of neutrophil recruitment: targeting the IL-8-CXCR1/2 chemokine axis limits the recruitment and infiltration of neutrophils into the tumor microenvironment, mitigating NET accumulation and associated pro-tumorigenic effects. (C) Enzymatic degradation of extracellular NETs: administration of DNase 1 digests extracellular DNA scaffolds within NETs, disrupting the structural integrity and diminishing the immunosuppressive and pro-metastatic properties. (D) Disruption of cancer-NET signaling: inhibition of pattern recognition receptors, such as TLR4/9 or blockade of the DNA sensor CCDC25 impairs NET-induced oncogenic signaling in RCC cells, reducing tumor-promoting interactions. (E) Modulation of neutrophil phenotype: targeting the TGF-β signaling pathway promotes the reprogramming of tumor-supportive N2 neutrophils into anti-tumor N1 phenotypes, thereby enhancing local immune responses and suppressing tumor progression. (F) Combination therapy: integrating NET-targeted strategies with immune checkpoint inhibitors or other immunomodulatory agents offers the potential to overcome resistance mechanisms and potentiate durable antitumor immunity in RCC patients. CCDC25, coiled-coil domain containing 25; CXCR1/2, C-X-C chemokine receptor type 1/2; DNase 1, deoxyribonuclease 1; IL-8, interleukin-8; MPO, myeloperoxidase; NE, neutrophil elastase; NET, neutrophil extracellular trap; PAD4, peptidyl arginine deiminase 4; RCC, renal cell carcinoma; TGF-β, transforming growth factor-beta; TLR, toll-like receptor.

Metabolic reprogramming of RCC

NETs enhance glycolysis in RCC cells, supplying rapid energy and biosynthetic intermediates crucial for tumor growth. Additionally, NET-derived VEGF promotes angiogenesis, sustaining tumor metabolism[93]. NETs also reshape immune interactions within the TME, promoting immune evasion and therapy resistance. By fostering glycolysis and angiogenesis, NETs reduce cancer cell susceptibility to targeted therapies and chemotherapy. Furthermore, NETs facilitate RCC metastasis by remodeling the ECM and aiding intravasation[94].

Inhibition of the NET formation pathway

Preclinical studies have shown that NET deficiency, achieved through PAD4 or NE inhibition, reduces metastasis. PAD4 inhibitors, like CI-amidine and GSK484, demonstrate anti-metastatic effects like PAD4 knockout models[95]. NE inhibitors, such as GW311616A, effectively impair tumor cell adhesion in metastatic models. However, the short serum half-lives of these inhibitors limit clinical application, necessitating longer lasting formulations[96].

DNase-1, which degrades NET-associated DNA, shifts cancer cells from a mesenchymal to an epithelial phenotype, which reduces invasion. DNase-1 therapy has demonstrated tumor growth suppression in colorectal cancer and hepatocellular carcinoma models, while decreasing metastatic potential in breast and lung cancer[97]. Clinical trials are evaluating DNase-based therapies, including *Pulmozyme* and Oshadi DNase formulations, for cancer treatment (Registration nos. NCT00536952 and NCT02462265). In addition, DNase-1 enhances chimeric antigen receptor (CAR)-T therapy and improves ICI efficacy in colorectal cancer models[98].

Targeting tumor-induced NET formation

Targeting NET-inducing factors, such as IL-8, cathepsin C (CTSC), amyloid β, and CXCR1/2, may impede metastatic progression and enhance immunotherapy. Unlike PAD4 or NE inhibitors, these interventions selectively inhibit pathologic NETosis, while preserving essential neutrophil functions[99,100].

Degradation of NET structure

NET degradation at tumor sites, while preserving antibacterial function, presents an alternative therapeutic strategy. DNase-1, which is FDA-approved for cystic fibrosis, reduces excessive NET accumulation in inflammation[95,101]. Recombinant DNase 1 exhibits potent anti-metastatic effects but the short half-life necessitates long-term treatment. Nanoparticle-conjugated DNase-1 prolongs circulation time, improving therapeutic efficacy. Hepatotropic adeno-associated virus (AAV)-mediated DNase-1 gene therapy shows promise in colorectal cancer by reducing liver metastasis and enhancing anti-tumor immunity[97,102].

Blocking NET-cancer cell interactions

NETs not only create physical barriers but also engage cancer cells through specific adhesion molecules, which promote invasion. Key mediators include integrins, carcinoembryonic antigen cell adhesion molecule 1 (CEACAM-1), toll-like receptor 9, and CCDC25[95,103]. Blocking these molecules with neutralizing antibodies or genetic modification reduces tumor migration and metastasis. However, redundancy in interaction pathways complicates this approach. Targeting NET-mediated interactions in RCC through immunomodulatory agents or pathway inhibitors may represent a promising therapeutic avenue[104].

Targeting novel immune checkpoints in RCC

The therapeutic modulation of novel immune checkpoints offers a promising strategy to enhance immune responses in RCC. While immune checkpoints regulate immune homeostasis, tumor cells exploit these pathways to evade immune surveillance. Among inhibitory checkpoints, T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) has a key role in immune exhaustion[87,105]. TIM-3 is expressed on T cells, NK cells, and DCs. Upregulation of TIM-3 on PD-1$^+$ CD8$^+$ T cells correlates with a poor prognosis. TIM-3 blockade in RCC-derived tumor-infiltrating lymphocytes (TILs) enhances proliferation and IFN-γ production, indicating the potential for reinvigorating T-cell responses. Early-phase clinical trials combining TIM-3 and PD-1 inhibitors have demonstrated safety and antitumor activity, warranting further investigation in RCC[106].

Lymphocyte-activation gene 3 (LAG-3), another inhibitory checkpoint, binds MHC class II molecules to modulate T-cell responses. While expression of LAG-3 on CD8$^+$ T cells in RCC is lower than TIM-3, dual blockade with PD-1 enhances IFN-γ production in TILs, suggesting a synergistic effect. Early clinical trials using LAG-3 inhibitors with PD-1 blockade have shown disease stabilization in some patients, which warrants further evaluation[107,108].

T-cell immunoreceptor with Ig and ITIM domains (TIGIT), which is expressed on T cells and NK cells, suppresses immune

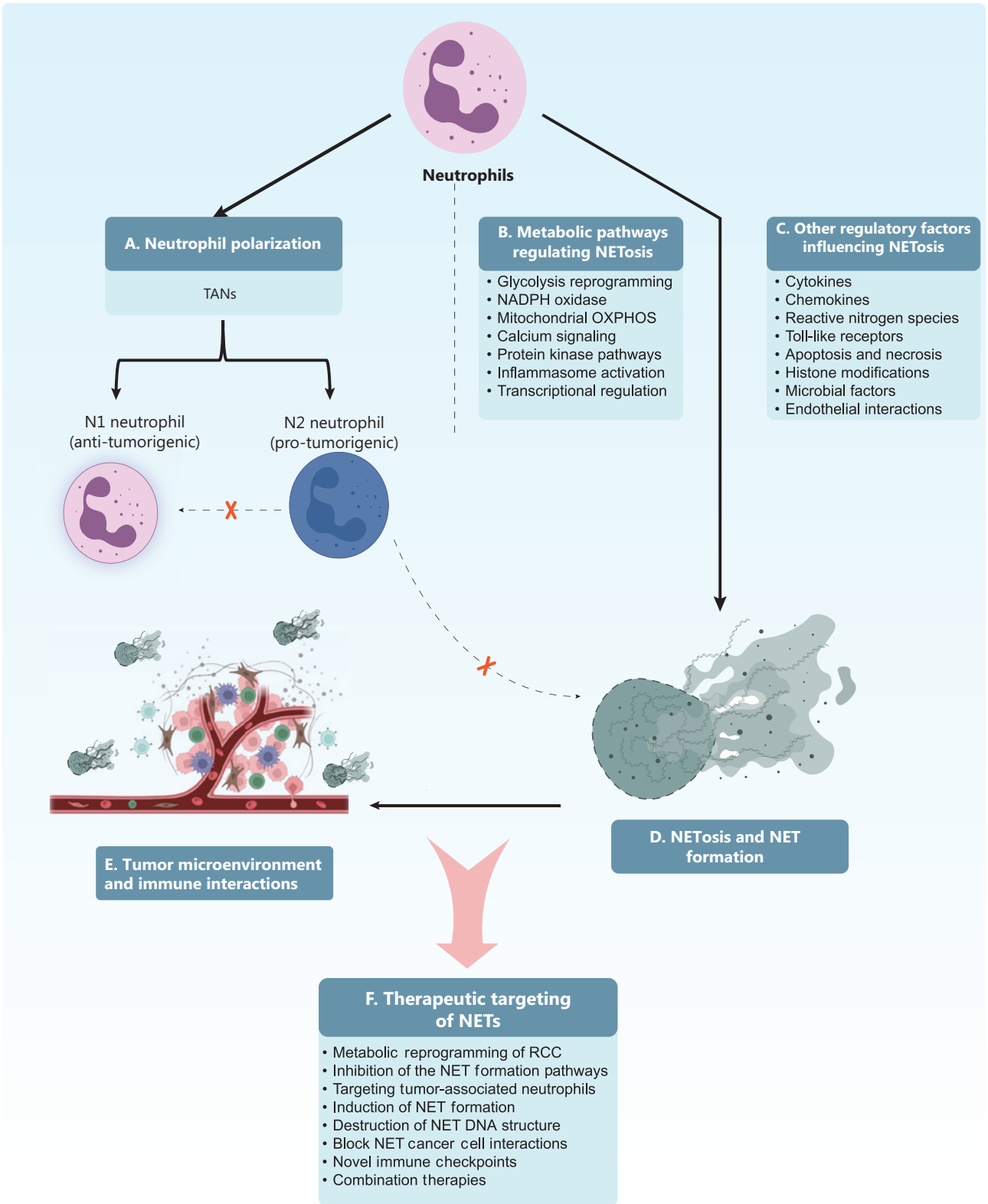
Neutrophils
A. Neutrophil polarization
TANs
N1 neutrophil
(anti-tumorigenic)
N2 neutrophil
(pro-tumorigenic)
B. Metabolic pathways regulating NETosis
• Glycolysis reprogramming
• NADPH oxidase
• Mitochondrial OXPHOS
• Calcium signaling
• Protein kinase pathways
• Inflammasome activation
• Transcriptional regulation
C. Other regulatory factors influencing NETosis
• Cytokines
• Chemokines
• Reactive nitrogen species
• Toll-like receptors
• Apoptosis and necrosis
• Histone modifications
• Microbial factors
• Endothelial interactions
D. NETosis and NET formation
E. Tumor microenvironment and immune interactions
F. Therapeutic targeting of NETs
• Metabolic reprogramming of RCC
• Inhibition of the NET formation pathways
• Targeting tumor-associated neutrophils
• Induction of NET formation
• Destruction of NET DNA structure
• Block NET cancer cell interactions
• Novel immune checkpoints
• Combination therapies

Figure 8 Neutrophil extracellular traps (NETs) in renal cell carcinoma (RCC): mechanisms, microenvironmental interactions, and therapeutic strategies. This schematic summarizes the multifactorial roles of NETs in RCC progression, integrating metabolic regulation, immune system interactions, and opportunities for targeted therapy. Distinct panels (A-F) describe sequential and interconnected processes, with arrows depicting mechanistic flow and potential therapeutic interventions. (A) Neutrophil polarization: TANs exhibit functional plasticity, capable of polarizing toward either anti-tumorigenic N1 or pro-tumorigenic N2 phenotypes depending on microenvironmental cues. N1 neutrophils facilitate tumor cell clearance by enhancing cytotoxic activity and supporting immune surveillance. In contrast, N2 neutrophils contribute to tumor progression through mechanisms, including immunosuppression, promotion of angiogenesis, and extracellular matrix remodeling. Within the RCC microenvironment, elevated levels of transforming growth factor beta, sustained hypoxia, and suppressive exosomal signaling can drive and stabilize N2 polarization. These conditions may limit phenotypic plasticity and render reprogramming toward the N1 state therapeutically resistant. (B) Metabolic pathways regulating NETosis: NET formation is tightly controlled by metabolic reprogramming within neutrophils. Key pathways include glycolytic shifts, NADPH oxidase activity, mitochondrial oxidative phosphorylation, calcium signaling, protein kinase cascades, transcriptional regulation, and inflammasome activation, all of which enhance the release of NET. (C) Other regulatory factors influencing NETosis: diverse microenvironmental cues further modulate NETosis, including cytokines, chemokines, reactive nitrogen species, toll-like receptor signaling, microbial products, apoptosis and necrosis-derived factors, histone modifications, and endothelial interactions. These pathways integrate with metabolic signals to fine-tune NET formation. (D) NETosis and NET formation: upon stimulation, neutrophils undergo NETosis, expelling decondensed chromatin decorated with histones and antimicrobial proteins into the extracellular space as NETs. These structures can trap pathogens but also promote cancer progression by supporting tumor cell adhesion, invasion, immune evasion, and angiogenesis. (E) Tumor microenvironment and immune interaction: within the RCC microenvironment, NETs interact with tumor and stromal cells, fostering an immunosuppressive niche. They reshape immune infiltration patterns, promote recruitment of suppressive myeloid populations, and facilitate metastatic dissemination by preparing pre-metastatic niches and supporting vascular remodeling. (F) Therapeutic targeting of NETs: strategies to disrupt NET-mediated tumor promotion include metabolic reprogramming of RCC cells, inhibition of NET formation pathways, targeting tumor-associated neutrophil recruitment and polarization, enzymatic degradation of NET DNA structures, blocking NET-cancer cell interactions, developing novel immune checkpoints, and combining NET-targeted approaches with other immunotherapies. NADPH, nicotinamide adenine dinucleotide phosphate; NET, neutrophil extracellular trap; OXPHOS, oxidative phosphorylation; RCC, renal cell carcinoma; TAN, tumor-associated neutrophil; TLR, toll-like receptor.

responses *via* interaction with poliovirus receptor (PVR) and Nectin-2[109]. Early-phase clinical trials of anti-TIGIT antibodies have reported tolerability and efficacy, particularly in non-small-cell lung cancer, prompting ongoing studies in RCC. In addition, killer-cell immunoglobulin-like receptors (KIRs) and V-domain immunoglobulin suppressor of T-cell activation (VISTA), are under investigation with VISTA inhibitors showing an early clinical promise[110].

Beyond inhibitory checkpoints, stimulatory immune checkpoints, such as 4-1BB and OX40, are being studied for the potential to enhance antitumor immunity[111,112]. These costimulatory receptors promote T-cell activation and proliferation with 4-1BB expression upregulated under the hypoxic conditions that are common in RCC. Early trials combining 4-1BB agonists with PD-1 inhibitors have shown safety and preliminary efficacy, which warrants further research. Overall, targeting novel immune checkpoints represents a promising strategy to augment immune responses in RCC with ongoing clinical trials poised to refine these approaches and improve patient outcomes[113,114].

Challenges and opportunities in targeting NETs and RCC-specific antigens

The development of anti-NET therapies remains largely dependent on xenograft mouse models, which fail to fully recapitulate the complexity of the TME in RCC patients. In addition, clinical trials evaluating NET inhibitors face significant challenges, including off-target effects, particularly in elderly cancer patients with compromised immune function. While targeting NETs is an emerging strategy in oncology, the immunomodulatory effects necessitate careful consideration in future therapeutic approaches.

A major limitation in RCC immunotherapy is the identification of tumor-specific antigens. Despite significant advances, only a limited number of RCC-specific antigens have been characterized, which restricts the scope of targeted therapies. A more systematic approach is required to expand antigen discovery efforts. Advances in immunogenomic technologies,

including mass spectrometry and single-cell sequencing, hold promise for the comprehensive characterization of the RCC "surfaceome." These approaches may facilitate the identification of novel targets for CAR-T therapies and antibody-based strategies. However, newly identified targets for effective therapies remain a challenge. Innovations in antigen prediction and detection, coupled with T-cell receptor (TCR) reconstruction and specificity testing, offer a potential solution by streamlining the link between tumor antigen discovery and the development of precision immunotherapies.

The RCC TME presents additional challenges and opportunities, particularly concerning immune evasion mechanisms. NETs contribute to an immunosuppressive milieu, promoting tumor progression and metastasis. The structural composition of NETs allows NETs to shield tumor cells from immune surveillance and enhances resistance to immunotherapies. Targeting NET formation or neutralizing their immunosuppressive effects may complement antigen-directed therapies, potentially enhancing the efficacy of CAR-T cells and TCR-based strategies. Addressing these challenges requires an integrative approach, combining antigen discovery with modulation of the TME, including NET-targeting strategies, to optimize RCC treatment outcomes.

Several NET-targeted agents are currently undergoing clinical evaluation. DNase-1 therapies, such as Pulmozyme (dornase alfa), are being repurposed for oncology with early-phase trials (e.g., Registration no. NCT02462265) exploring safety and immune-modulatory potential. PDA4 inhibitors, like GSK484, which have shown promise in preclinical models, face translational challenges due to short half-lives and lack of cancer specificity. Similarly, elastase inhibitors have shown efficacy in reducing metastasis *in vivo* but have encountered pharmacokinetic limitations in clinical contexts. Moreover, the risk of compromising host defense mechanisms remains a significant concern. Combination strategies, such as pairing DNase-1 with ICIs, are being investigated to improve therapeutic efficacy while minimizing systemic immunosuppression.

Conclusions

Recent advances underscore the role of tumor-infiltrating immune cells in RCC progression and the metabolic reprogramming of fatty acid oxidation, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway (PPP), and glutaminolysis. Nutrient deprivation within the TME limits anti-tumor N1 neutrophils, favoring the activation of pro-tumor N2 neutrophils, which further supports tumor progression. Metabolic manipulation is emerging as a viable therapeutic strategy for RCC and is summarized in **Figure 8**. Despite progress in immunotherapy, the scarcity of tumor-specific antigens remains a major limitation. Advances in HLA-restricted antigen prediction and TCR engineering are improving T cell-targeting specificity, yet challenges persist in overcoming immune evasion mechanisms. NETs have a critical role in RCC progression by modulating immune responses, highlighting the need for strategies to neutralize the effects. However, the interactions between NETs and RCC-related processes, including inflammasomes, autophagy, and metabolic reprogramming, remain poorly understood. Future research must focus on elucidating these complex interactions within the TME to refine immunotherapeutic strategies and improve clinical outcomes in RCC.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Qiao Zhang, Zhe Yang, Ting Guo.
Collected the data: Asif Shahzad, Yueli Ni, Zhouran Teng, Wenjing Liu, Hinggang Bai, Xiangjie Liu, Kun Cui.
Contributed data or analysis tools: Qiuxin Duan, Yijian Sun, Zhe Xu, Rong Che.
Performed the analysis: Asif Shahzad, Zhe Xu, Jinshan Zhang, JiaoJiao Xia.
Wrote the paper: Asif Shahzad, Qiao Zhang.

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