Metabolic Regulation of Natural Killer Cells

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Abstract. Natural Killer cells, known as NK cells act crucially in protection of anti-tumor and anti-virus activities as innate lymphocytes. Due to its unique characteristics such as recognizing and attacking stressed cells independently, and bridge connection function between innate immunity and adaptive immunity, it possesses profound potential and prospect in immunotherapy. However, due to resistance existing in combination of therapies and undeclared metabolism pathways, its application in clinical trials has been underestimated. Metabolic configurations are adopted to support functions, which is dynamic when immune cells experience the procedure of metabolic reprogramming in responses. In order to support anti-tumor functions, NK cells up regulate both glycolysis and OXPHOS, leading to the alternative of energy metabolism structure and ATP relief upon activation with pro-inflammatory cytokines. After activation, what exactly determines phenotypes and destiny of NK cells is also being considered as the classification standard of receptors distribution is found both vague and not universally applicable. Additionally, author focus on metabolic factors in solid cancer treating, which are highly relative to tumor microenvironment (TME). TME provides many molecules for the prevention of potential effector function of normal cells (especially immune cells) and protection of tumor. For example, substances such as TGF-β are capable to not only downregulate NK cell cytotoxicity and cytokine secretion but also reduce the extent of metabolism and proliferation, and also induce effector NK cells to increase the production of ILC1-like characteristics. This review will discuss NK cells' metabolic foundation to realize NK cell effector functions and analyze the relationship between phenotypes, NK cell fates and metabolism. Also, basing on impaired functions, immune suppression and resistance of joint therapies, the author will discuss different pathways and possible treating targets and therapies in order to boost cancer treatment progress such as adoptive NK cell therapy, bio- or tri- engager therapy and CAR-NK therapy.

Keywords: Glycolysis; mitochondrial metabolism; metabolic regulations; phenotypes.

1. Introduction

NK cells are hardwired to identify stressed cells, eliminate tumor cells and control viral infections by different means, are highly considered in cancer treatment in immunotherapy. As great strides have been made, their widespread identification in spite of neoantigen presentation as well as improved efficacy against cancers that lost MHC1 due to developed resistance mechanisms demonstrate the tremendous promise of immunotherapy in the treatment of cancer. The result is therapies include mobilizing NK cells produced by patients themselves with therapeutics, providing a number of alternative NK cells as adoptive therapeutical populations ex vivo, bi- or tri- specific engages and antibodies have been introduced. Due to its similarities compared to T cells and the remarkable progress of CAR-T therapy, CAR-NK therapy has also entered people's vision and the stage of clinical trials. Although researches are booming, the efficacy treating solid tumor is still unsatisfied and results have revealed further studies are needed.

One of those limitations regarding NK cell functions is the immunosuppressive tumor microenvironment (TME) where tumorous and other immune cells generate the proper conditions for the growth of tumor while limiting the activation. In the TME, they receive cytokines and factors from tumor and associated cells resulting down modulation of activating receptors and also receive signals from inhibitory receptors which is determinant for the activation of NK cell [1]. Local metabolite availability participates shaping tumor cell phenotypes and anti-tumor immune responses and associates with therapeutic sensitivity [2] which encourage immunologists to work on immunometabolism. Immune cells response rapidly when the body is confronted with abnormal

interference such as infection and inflammation, exerting immune function, eliminating target substances and maintaining homeostasis. Metabolic changes may occur in response to signals received from other cells and alter of environment and in activation from a relatively static state. At molecular level, the life activities of cells depend on energy supply and synthesis of necessary substances; at cellular level, the realization of immune function is separable from the metabolic pathways involved in cell growth, division, differentiation, maturation and activation.

2. Metabolic Factors

Relying on metabolic pathways, NK cells cater their effector functions requiring energy consumption and nutrients for anabolic synthesis. Here, we only discuss energy consumption and mitochondria metabolism.

2.1. Metabolism Oxidizing Glucose

2.1.1 Glucose Roles

Although glutamine and fatty acids are significant sources of energy (not yet known what role they play), it is also known that glucose is the most essential origin in cellular metabolism. Hallmarks of TME include hypoxia, chronic inflammation and immune suppression. For better solid tumor treatment, GLUT (a glucose receptor) inhibitor Glutor has been tested to eliminate the impairment of poor nutrient environment. Through inhibition of glucose uptake without affecting cytotoxicity, NK cells treated by Glutor for a quite longer time period are declared hopeful in clinical trials [3].

Glycolysis, the TCA cycle and OXPHOS are three main glucose catabolisms to acquire ATP and realize biosynthetic intermediates.

The correlation between NK cell metabolism and its function is achieved in four ways: (1) providing energy; (2) metabolic intermediates serving as important signaling molecules; (3) as a carbon source for biosynthesis preconditions; (4) having a direct role in controlling immune signals and immune cell effector functions.

2.1.2 Glycolysis, TCA Cycle and OXPHOS

Upon activation, immune cells exhibit high demands for energy and nutrients especially the anabolic synthesis to ensure their further functions. It is oxidative phosphorylation and glycolysis that supply energy for cells as two major metabolic pathways. On the one hand, through glycolysis, glucose is transferred into pyruvate by several metabolic activities which is independent with oxygen, as a relatively less efficient way of ATP generation compared with OXPHOS. On the other hand, in the process of biological oxidation, substrate dehydrogenation produces NADH and FMNH2, which are transferred through the respiratory chain to oxidize and generate water [4]. Then, the released free energy is used to couple ADP phosphorylation to ATP whose coupling effect between oxidation and phosphorylation is called oxidative phosphorylation. But in pro-inflammatory cells and cancer cells, glycolysis is reported to be the predominant metabolic route, presumably since it could be quickly activated through glycolytic enzymes and because it can supply intermediates for cellular biosynthesis [3]. Recent research has shown that OXPHOS is mostly necessary for the survival of resting NK cells. Both murine and human NK cells up-regulate glycolysis and OXPHOS after being activated by cytokines like IL-2 to support IFN-production, whereas inhibitory cytokine TGF-, which is frequently present in the TME, showing suppression of NK cell metabolism and functions [5]. It demonstrates that human NK cells respond to anti-CD16 antibodies and NKG2D ligand stimulation by increasing glycolysis and OXPHOS, and that suppression of either glycolysis or OXPHOS dampens NKR-induced IFN- production. Additionally, it has been discovered that glycolysis, which promotes the degranulation of NK cell and the expression of Fas ligand, is a major factor in NKR-induced NK cell cytotoxicity [6]. A crucial factor determines the alter of metabolic pathways from glycolysis to OXPHOS is oxygen, which is of profound significance in the realization of NK cells effector function. However, in TME, situation is not that optimistic. The lack of oxygen inhibits

OXPHOS which impairs activated NK cells and blocks the effector action which is a vital factor in NK cell therapy resistance. The supplement oxygen available into tissue far from vessels is limited as a result of the tumor vasculature's rapid expansion, increasing the need for oxygen in tissues that are proliferating. Most solid tumors have persistent hypoxia, which is a diffusion-limited oxygen shortage. Furthermore, another more severe type of hypoxia might be caused by the temporally reduced blood flow by the tumor vasculature due to its chaotic and unpredictable nature [5]. Metabolic changes brought on by hypoxia are characterized by enhanced glycolytic metabolism in hypoxic tumor cells [7].

2.1.3 Glucose Restriction and Lactate Metabolism

As previously indicated, when there is a metabolic stress, cancerous cells are competing with NK cells for the restricted glucose supply, and tumor-driven glucose limitation could decrease the glycolysis of NK cells, reducing their ability to fight tumors [8]. Evidence suggests that the TCA cycle in nearby cancer cells can be powered by lactate produced by the anaerobic metabolism of glucose in hypoxic tumor areas [9]. It has been shown that lactate has an impact on cancer cells, which is changing the way that energy metabolism is studied. However, exposing in a high level of actate impairs the effective function of liver-resident NK cells and also causes mitochondrial malfunction and death [10]. Although lactate buildup in the TME has been proposed as a candidate for targeted anticancer therapy, with these findings in clinical therapy will be extremely difficult [11]. More research is required, as a thorough examination of lactate metabolism signaling and the interaction of lactate with other elements in the TME, particularly NK cells, could be a viable way to get around immunotherapy's drawbacks [8].

2.2. Mitochondrial Metabolism

2.2.1 Pathways

Metabolized to mitochondrial acetyl-CoA and pyruvate, glucose is capable to yield decreased NADH. Acetyl-CoA is combined with oxaloacetate in the process of mitochondrial citrate generation, which is later transported with the help of the citrate-malate antiporter SLC25A1 [1]. Inside the cytosol, ATP citrate lyase metabolizes the citrate, and acetyl-CoA and oxaloacetate are generated, which can further be transported to malate and during this process, oxidizes NADH was oxidized and NAD+ was yield [1]. Malate re-enters the mitochondria via SLC25A1, where it is converted, generating a second NADH molecule [1]. By reacting with another glucose-derived acetyl-CoA, oxaloacetate is capable to generate other molecules of citrate, promoting the cycle. However, the citrate-malate alter also generate additional acetyl-CoA that serves as substrates for acetvlation process or lipid synthesis [1]. It has been shown that two groups of cells responded in a dichotomous manner to activation, with NK^{Bright} cells exhibiting additional mitochondrial potentiation and NK^{Dim} cells exhibiting paradoxical mitochondrial fission and depolarization [12]. Inhibiting mitochondrial fragmentation was able to reverse the latter result, which reduced interferon- production and suggested that mitochondrial polarization is a key factor for the regulation of NK cell activity [12]. NK^{Dim} cells are diverse, and mitochondrial polarization has a significant impact on both improved survival and function.

On the other hand, because patients with mitochondrial fusion genetic defects lacked adaptive NK cells, it demonstrates limited survival ability in vitro. All of these researches indicate that mitochondrial polarization is the main regulator of mature NK cell fitness. In addition to NK cells, research indicates that NFAT activity and T cell signaling are both modulated by mitochondrial metabolism, which is extremely encouraging for the application of NK cell therapeutics.

2.2.2 Significance and Memory Function

The OXPHOS inhibition is shown to relieve hypoxia by pressing oxygen demand in preclinical studies, but is not yet proven in the clinic and can only be beneficial to patients disturbed by tumor hypoxia due to an increased oxygen consumption. This increases the effectiveness of both radio- and immunotherapy, and also their combination. Additionally, the immunosuppressive effect brought on

by the TME's acidity will not be abolished by OXPHOS inhibition and may instead be exacerbated by doing so. This effect is also linked to both the efficacy and resistance to immunotherapy. As oxidative metabolism is not completely related to tumor hypoxia, further discoveries of cellular metabolism and development of personalized medicine are highly expected.

Not only in cancer treatment, mitochondrial metabolism also alters in antivirus activity relating to NK cells' memory. In an experiment, MCMV infection causes NK cells that express the activating receptor Ly49H, which binds to the MCMV-specific viral ligand m157, to proliferate quickly, resulting in mitochondrial fitness. Reduced mitochondrial membrane potential and elevated amounts of reactive oxygen species are two characteristics. These virus-specific NK cells survive after the contraction phase as a self-renewing regulation of memory NK cells with improved capability to response with reactivation. One vital process through the procedure to memorize is storing mitochondrial fitness longer, realized by replacing damaged mitochondria. To increase numbers of memory NK cells, pharmacological approaches are introduced to realize the activation of protein kinase. Interestingly, similar roles are discovered in the trials to control mouse T cells' memory.

Due to the tight connection between memorizing function and mitochondrial metabolism, it is considered that whether the function-based phenotype classification is more relying on the pathways and percentage of different metabolic pathways.

3. Metabolic Difference Between NK Cell Phenotypes

According to information of oxidization metabolism above, it has assumed the tight relationship between NK cell effector function and metabolic activities. Due to the realization of NK cell effector function is somehow relied on receptors expressed on membrane, previous studies prefer classify the phenotypes by recognizing types and numbers of receptors on the membrane which is related to specific functions but phenotypes cannot determine their fate. The following part will discuss the metabolic classification standard in distinguishing phenotypes of NK cells and their fates related to their metabolic reaction.

3.1. Cytotoxic NK Cells

Cytotoxic NK cells are known in attacking stressed cells and are fueled mainly by glycolysis. After activated, cytotoxic NK cells promote percentage of glucose-driven glycolysis and OXPHOS which can better enhance cytotoxicity. It possesses greater ability of glycolysis and OXPHOS with greatest cytotoxicity.

3.2. Regulatory NK Cells

Although all NK cells are expected to possess great cytotoxicity, signals in TME can polarize NK cells to regulatory phenotype to support the development of tumor and inhibit other cytotoxic cells. Additionally, regulatory NK cells exhibit crucial homeostatic characteristics in tissue growing and immune tolerating especially in fetal tolerance in pregnancy, placental development, immune cells in the liver and also regulation of fibrosis [13].

Compared with cytotoxic NK cells, regulatory NK cells are polarized to their destiny under hypoxia and limited glucose conditions, so glycolysis and OXPHOS are less active. Additionally, regulatory NK cells also rely on other fuels such as fatty acids or amino acids which can be distinguished from cytotoxic NK cell at a metabolic level. However, clear boundaries such as unique metabolic pathways to differentiate cytotoxic NK cell and regulatory has not been declared yet.

3.3. Memory NK Cells

As mentioned, memory NK cells can memorize stimuli information and can be stimulated with greater response when antigens attack again. Secondary response includes adaptive-like antigen-specific one and innate-like non-antigen-specific one which is depended on sensitizing stimulus. What's more, although memory capacity is discovered also in cytotoxic NK cells and regulatory NK

cells, their memorizing function is more rapid and enhanced. Basing on the ability, memory NK cells reveal a vital metabolic hallmark equipping themselves to exhibit enhanced mitochondrial fitness including undergoing autophagy to clear dysfunctional mitochondria, exhibiting an increased SRC, reduced of ROS and reduced membrane potential. When it comes to energy source, they maintain a steady level of glucose metabolism and up-regulate genes expression related to lipid metabolism. The diversification in fuels may match the hallmark of longevity and great energy demands.

4. Metabolic Changes in Cellular Processes

4.1. Metabolism during NK Cell Development & Activation

After NK cells' developing and growing, metabolisms rise in NK cells that not mature enough to benefit proliferation. Among the procedure, what should be emphasized on is the relationship between glucose transporters expression, percentages of glycolysis and the speed of reaching maturation and realization of self-tolerance [14].

Multiple combinations of cytokines promote the regulation of energy basis metabolic activities in these NK cells accompanied by nutrient transporters, mitochondrial mass and so on related to regulating the environment.

4.2. Phenotypes and Functions as Aging

As people age, studies have shown that both the absolute quantity of circulating NK cells and the distribution of NK cell subsets changed. Most studies show an elevation in the number of NK cell as aging, despite some debate in the field. In comparison to the younger group, results from various approaches have suggested an elevation in expanded late lower cytotoxic fractions, enhanced apoptotic signaling and diminished anti-viral defense.

It has discovered a decline in the production of cytokine or chemokine per cell with aging, which is thought to be related to the transition of NK cells from CD56bright to CD56dim with aging. The former is in charge of creating these mediators. Additionally, it is well known that NKCC declines with age and that this loss is correlated with both an increase in the prevalence of infectious illnesses and a clear decline in the cytotoxic efficacy of NK cells. It is noteworthy, however, that one study found a correlation between age-related increases in cytotoxic activity and a change from the CD56+57 to the CD56+57+ phenotype. Due to receptors' contribution to target cell recognition, activating receptors such as NKp30 and NKp46 have reported reduced while inhibitory receptors such as KIRs have reported rose which result a change in the capacity to accept pro-activation signaling, which could be a symptom of a general shift towards the inhibitory cellular phenotype. Additionally, it has been suggested that a decrease of Perforin expression in NK cell is the mechanism behind the age-dependent declining in cytotoxic NK cell. Collectively, these findings show that biological alterations in NK cells from older patients affect their interaction both with other immune cells and target cells. One explanation for the elevated number of cytotoxic NK cell is that it could be an effort to make up for the age-related decline in effector activity at the level of the individual NK cell. Signal transduction and aging systemic milieu are also reported relative. Due to similarities comparing to T cells, whether NK cells experience a similar pattern of exhaustion is discussed. More research is required to evaluate whether telomere attrition actually has any functional consequences on cytotoxicity, despite some evidence pointing to it as a alternative cause of NK cell function decline with aging. Neuroendocrine factors are seemed to dominantly inhibit NK cell cytotoxicity and cytokine production as well as the engagement of growth factors and receptors [15].

4.3. Homeostatic Functions and Balance

In addition to anti-tumor and anti-virus functions, NK cells also possess homeostatic characteristics that can balance the ability to clear and tolerate. Pairing with other molecules is capable to enhance tolerating ability during pregnancy and takes part in the invasion of trophoblasts and so on, which is beneficial for both mothers and children.

5. Conclusion

This study tried to find out concept that how supportive and what extent of metabolism is in dysfunction of NK cells. Energy source and oxygen are two of the most vital factors in oxidation of the combination of glycolysis, OXPHOS, lactate metabolism and mitochondrial metabolism. Besides, substance such as NAD+ can regarded as an important factor to connect cell metabolism and signal transduction which suggests a possible possibility for NK cell-based immunotherapy. Currently, CAR-NK therapy and adaptive NK cell therapy are regarded as two most promising ways to treat cancer in NK cell therapy. Numerous mitochondrial abnormalities, such as altered mitochondrial structure, were seen in the patients' NK cells, and they were unable to respond to cytokine stimulation by increasing glycolysis and OXPHOS metabolic rates. However, shared drawbacks including poor availability and persistence in vivo drives the result far from assumptions, which is associated with metabolic activities. Optimizing this route may capable to increase the range and effectiveness since OXPHOS and mitochondrial fitness encourage longer survival and enhance cytokine output released by NK cells. In the future, we are expecting further exploration of uncleared pathways to better illustrate immune suppression and cross-resistance existing in current NK cell-based therapies. More solutions can be foreseen to tackle difficulties such as optimizing current adoptive NK cell therapy such as broaden the source of transfer and increase cytotoxicity and persistence on new therapies like bi- or tri- antibody engagers using techniques including gene editing or artificial intelligence and so on. All findings reveal the bright future of metabolic treating clinic, suggesting to provide potential strategies for immunotherapy.

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