The research progress on epigenetic control of CAR-T therapy

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Abstract: Epigenetic regulation of gene function is closely related to the development of cancer. The ability to reprogram the epigenetic landscape in the cancer epigenome is one of the most promising targeted therapies for treatment and reversibility of drug resistance. Epigenetic changes in cancer development and progression may underlie individual differences in drug responses. Over the past decade, our understanding of cancer epigenetics has made significant advances, particularly in abnormal DNA methylation, microRNA (miRNA) and non-coding RNA abnormalities, and altered histone modification states. Evaluation of the main cancer epigenome revealed that almost all cancer had abnormal methylated genes and altered miRNA expression. This review focused on the epigenetic control of cancer therapy and summarized the main regulation way of CAR-T therapy development.

1. Introduction

Gene silencing refers to regulating gene expression in cells to prevent the expression of a particular gene. Furthermore, gene silencing is generally considered the same as gene knockdown. Because when genes are silenced, their expression decreases [1, 2]. In comparison, when genes are knocked out, they are completely deleted from the genome and therefore are not expressed anymore [2]. Gene silencing is considered a gene knockdown mechanism because methods used to silence genes, such as RNAi, CRISPR, or siRNA, usually reduce gene expression by at least 70% but do not eliminate it. Methods using gene silencing are considered better than gene knockout because they allow researchers to study essential genes that animal models need to survive and cannot be removed. Moreover, they can provide an overall view of the disease's development because it is usually associated with the reduced expression of genes [1].

Transcriptional gene silencing (TGS) belongs to the transcriptional type of gene silencing and occurs before the transcription of genes. TGS often refers to gene silencing caused by things like DNA modification and chromosome heterochromatinisation that inhibit the gene's normal transcription. In mammalian cells, cells can be manipulated by introducing transgene delivered as DNA or by a virus vector. For example, transgenetic mice are created by microinjection of DNA directly into fertilised eggs of mice. On the other hand, the gene transfer into a human is often through DNA transfection or by infecting the cells with a virus vector. The integration of transgenes into the genome is random. They can approach or integrate into heterochromatic regions, thereby preventing transcriptional activation. This may result in partial or complete transgene silencing, which is called positional effect (PE). PE is usually caused by differences in the epigenetic organization of genomic regions and can be sub-categorised according to the pattern of transgene expression. If transgene silencing is completed in all cells of a specific tissue, it is called stable PE. Stable PE is sometimes characterized by cell cycle-dependent regulation of transcription, the cell timing position effect (CTPE). CTPE results from chromatin configuration, which affects transcription in all tissue cells, but only during part of the cell cycle. Compared to stable PE, if transgene silencing is variable from one cell to another in a cell population, it is unstable PE, especially position-effect variegated (PEV). PEV causes restriction of gene activity in a subset of cells from a homogeneous population. Silence is set randomly and can be inherited later. The occurrence of PEV is consistent with cell-to-cell differences in the ability of heterochromatin to invade specific transgenes located in nearby euchromatin regions. This variable

ability to invade euchromatin seems to depend on the local concentration of heterochromatin (inhibitory) and euchromatin (activator) proteins. It is speculated that these two groups of proteins participate in the local competition to inhibit or activate transgenic chromatin. Changes in the concentration of inhibitory or activating proteins can lead to modification of PEV.

Post-Transcriptional Gene Silencing (PTGS) is the gene silencing that takes place after transcription. RNA interference (RNAi) belongs to this type of gene silencing. RNAi is the efficient degradation of mRNA induced by the double-stranded RNA (dsRNA) phenomenon and then cause gene silencing that happens after transcription of genes. Micro RNAs (miRNAs) are a type of small RNA. Furthermore, most miRNAs comes from the RNAs transcribed in the nucleus of cells. These RNAs then fold and are transported to the cytoplasm as double-stranded precursor miRNAs. Double-stranded precursors of miRNAs and small interfering RNAs (siRNAs) then bind to an enzyme known as DICER to form an RNA-induced silencing complex (RISC). Moreover, DICER is an endonucleic protein that can cut RNAs into short segments. Then the short dsRNAs bind to an Argonaute protein, theoretically, the short segments produced by DICER means that there are two single-stranded RNAs available, whether they are siRNAs or miRNAs, but actually, only one from the two RNAs are used as guide strand and remains bound with Argonaute. Furthermore, they will guide the binding between Argonaute and specific messenger RNA (mRNA) through the complementary base pairing mechanism. Once bound, the Argonaute will enzymatically catalyse the cleavage of the target mRNA sequence. The cleaved mRNA then becomes non-functional and hence is "silenced".

2. DNA methylation cancer

Epigenetics is the heritable phenotype changes that do not involve the alteration of DNA sequence. Mutations in oncogenes usually result in the gain of function, while mutations or deletions related to tumour suppressor genes will result in the loss or inactivation of negative regulators. However, loss of function can also occur through epigenetic changes, such as DNA methylation. DNA methylation is currently the most studied epigenetic modification in mammalian cells. In normal cells, it assures the proper regulation of gene expression and stable gene silencing. Therefore, normal and appropriate DNA methylation are essential for proper cell functions. The abnormalities and malfunctions in this process can lead to various diseases, including cancer. DNA methylation is basically the adding of methyl groups to DNA that will alter the activity of gene segments without changing the sequence of DNA. When DNA is methylated in the promoter region of a gene that initiates transcription, the gene is inactivated and silenced. However, this process is often dysregulated in tumour cells. In cancer, epigenetic silencing caused by methylation is generally thought to be at least as frequent as mutations or deletions, which results in abnormal silencing of normal tumour suppressor functions [3, 4].

DNA methylation is regulated at multiple different levels in both normal and tumour cells. The addition of methyl groups to DNA is carried out by a family of enzymes called DNA methyltransferases (DNMTs). In addition, the chromatin structure near the gene promoter region can also affect DNA methylation and transcription activity. These mechanisms are regulated by various factors, such as nucleosome spacing and histone acetylase, which affect the acquisition of transcription factors [5].

As mentioned above, DNMTs are enzymes that can catalyse the addition of methyl groups to cytosine residues from DNA. DNMTs can be divided into de novo methyl transferases and maintenance methyl transferases. De novo methyl transferases can recognise things in the DNA that enables them to methylate cytosines newly so that they can establish new methylation patterns. On the other hand, maintenance methyl transferases are responsible for adding methylation to the DNA when one strand of the DNA is already methylated, and this shows that the primary function of maintenance methyl transferases is to maintain and repeat the methylation patterns established by de novo methyl transferases. The DNMTs found in mammalian cells include DNMT1, DNMT3a and DNMT3b. In mouse developments, DNMT1 seems to be maintenance methyl transferases that are responsible for maintaining the methylation patterns, while DNMT3a and DNMT3b appear to be de novo methyl transferases that mediate the establishments of methylation patterns [6, 7]. Furthermore, the abnormal

gene hyper methylation may not be maintained by DNMT1 alone, in which case DNMT1 and DNMT3b are assumed to cooperate to accomplish this function.

Although methylation can control gene activity, methylation alone is not sufficient to inhibit transcription. Local chromatin structure can also assist in determining whether a gene is transcribed or repressed. For example, DNMT can regulate gene silencing accompanying promoter DNA methylation by recruiting histone deacetylase (HDAC) and other chromatin-binding proteins to the promoter site to maintain histone deacetylation as the histone acetylation status is important for regulating chromatin structure and gene transcription. The chromatin structure surrounding unmethylated and transcriptionally active genes is different from that of methylated and silent genes. Thus, both nucleosome structure and histone acetylation affect chromatin structure, thereby regulating gene transcription. It can be concluded from the above that histone acetylation and methylation act synergistically to regulate gene transcription (Figure 1).

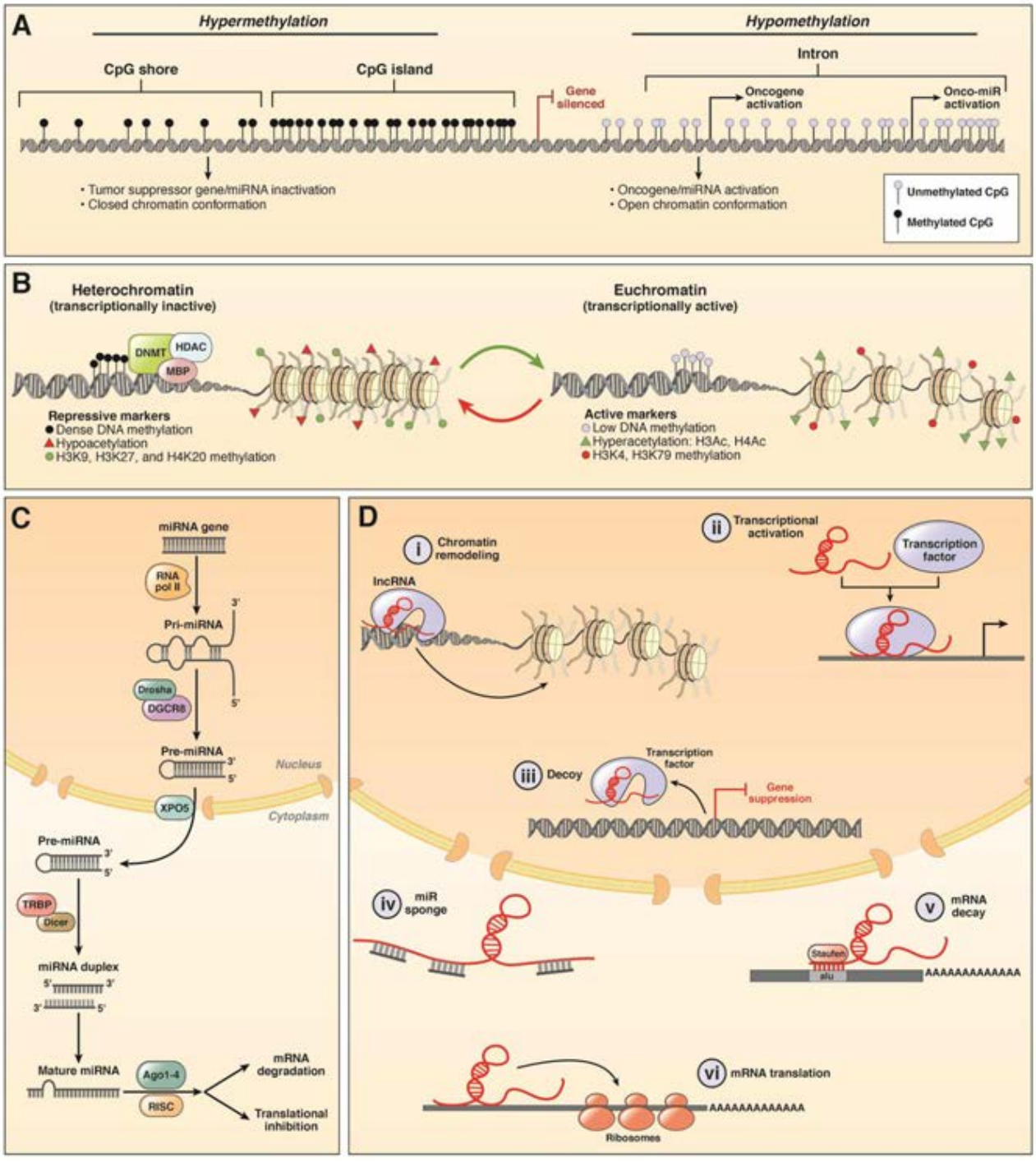

Figure 1. The mechanism of epigenetic control of cancer therapy [4].

Histone acetylation is required to maintain chromatin in an open and transcriptionally active state. This allows transcription factors, histone acetylases, and other regulatory co-activators that promote gene expression to combine. In contrast, the role of HDAC is to deacetylate these residues, thereby maintaining transcriptional silence. The binding of HDAC to hyper methylated chromatin is guided by DNMT and methyl cytosine binding proteins, forming complexes with other regulatory proteins to achieve the blockage of access of transcription machinery to the promoter. HDACs are an indispensable part of maintaining transcriptional silencing, and they can increase gene expression in the situation without hyper methylation. A variety of HDAC inhibitors have been studied and proved to be effective in inhibiting the deacetylation of histones in human tumour cells, leading to the accumulation of acetylated histones. In leukaemia cells, treatment with HDAC inhibitors can lead to growth arrest, differentiation, or apoptosis. These drugs are expected to be used in cancer treatment, especially when combined with methylation inhibitors, other differentiation agents, or cytotoxic compounds. It should be further explained that methylation plays a leading role in histone deacetylation, so transcription will not occur if methylation is not suppressed first. Therefore, initial treatment with azacitidine or decitabine, followed by the use of HDAC inhibitors, can produce additive or synergistic effects on the re-expression of transcription-silencing genes.

The widely accepted "second hit" hypothesis of carcinogenesis proposed by Knudson considered that malignant transformation requires the loss of function of two alleles in a given gene. The first hit is usually a mutation in a critical gene, and then the wild-type allele is lost through deletion or loss of heterozygosity. This first blow may occur in familial cancers through germline mutations, while in non-hereditary sporadic cancers, somatic mutations are more common. Subsequent deletion, point mutation, or loss of heterozygosity results in the loss of the remaining alleles to eliminate the remaining functional genes. Inactivating regulatory genes in this way can lead to the development of cancer. Similar results can be achieved by the inactivation of epigenetic genes caused by abnormal promoter methylation. In cancer cells, methylation in the promoter turns off key genes that may inhibit tumorigenesis. For example, tumour suppressor genes and those encoding cell adhesion molecules and growth regulatory proteins are usually silenced by DNA hyper methylation in hematopoietic malignancies [8-10]. In this case, the hyper methylation of the promoter region can establish the initial hit in many somatic cancers with subsequent deletion or mutation that will eliminate the second gene copy. This epigenetic alteration also appears to induce the second hit in familial cancers. Furthermore, the hyper methylation of both alleles has been noticed in nonfamilial tumours which mutations and other genetic mechanisms are not present. Since the methylation of CpG islands rarely occurs in normal cells, methylation provides a selective tumour-specific therapeutic target. At the same time, compared with mutant genes, the DNA sequence and protein products of methylated genes remain unchanged. Therefore, pharmacological inhibition of methylation-mediated suppression can derepress the inappropriately silenced genes and restore normal gene function.

The demethylating agent azacitidine and its deoxy derivative decitabine are widely known and concerned as potent inhibitors of DNA methylation. Preclinical studies have shown that after cellular uptake and phosphorylation, azacitidine binds to RNA, inhibits RNA synthesis and has cytotoxic effects. After ribonucleotide reductase is converted to 5-aza-2' deoxycytidine diphosphate and subsequently phosphorylated, the triphosphate form is integrated into the DNA to replace the natural base cytosine. Since the 5'nitrogen atom replaces the carbon, DNMT is trapped on the substituted DNA strand and inhibits methylation. Therefore, in the presence of these analogues, a large part of DNA becomes hemimethylated. The second round of DNA synthesis in these drugs resulted in complete demethylation of double-stranded DNA. Although both azacitidine and decitabine can inhibit methylation, they are indeed different in some respects. Because it contains a ribose moiety, azacitidine can be phosphorylated by uridine-cytidine kinase, and when this happens, it will then be integrated into RNA. Since decitabine contains a deoxyribose group, it can only be incorporated into DNA [10]. Although some people believe that the difference in the incorporation of RNA and DNA may explain the difference in methylation and toxicity characteristics between the two drugs, there is no direct clinical data to support this proposal [11].

3. Histone modification cancer

The primary component of chromatin is the nucleosome, formed by 147 DNA base pairs wrapped around the histone octamer. There are five forms of histones: histones H1, H2A, H2B, H3, and H4 [12] and several histone variants, which exist in a subpart of the nucleosome and participate in specific functions. Examples include DNA repair and gene activation [13]. The octamer structure of the nuclide consists of H3-H4 tetramers flanked by H2A-H2B dimers. The N-terminal tails of these "core" histones undergo methylation, acetylation, phosphorylation, SUMO acylation and ubiquitination, and is modified in these processes. Together with DNA methylation, histone post-translational modification is the main effector of epigenetic control of cell function and has been shown to have core functions in regulating gene expression, DNA replication and repair, cell division, and apoptosis [14].

Similar to DNA methylation regulation, histone acetylation can be regulated through the opposing actions of histone acetyltransferases (HATs) and HDACs, while histone methylation is due to histone methyl transferase (HMT), protein methyl transferase (PRMT) and demethylase. Researcher have shown that the HAT function changes caused by mutations, translocations, or simple overexpression are related to many diseases, including cancer. In addition, HATs have been found to have multiple roles in cancer research because they can act as either tumour suppressor genes or oncogenes, depending on genetic content and specific situation. The studies on mice show that p300/CBP is the tumour suppressor gene, and the deletion or mutations in p300 is found in many cancer samples, including colorectal, gastric, and epithelial cancers. At the same time, HATs can also act as oncogenes. Down-regulation of p300 activity can lead to growth inhibition and activation of senescence checkpoints in human melanocytes.

The above data and evidence powerfully illustrate the critical position of HAT in the development of drugs and therapies, and its characteristics also determine that it can play different roles in different types of tumour cells and different apparent modifications. Therefore, a variety of HAT inhibitors have been developed in recent years, and the HAT inhibitors developed so far can be classified as natural or synthetic compounds.

Anacardic acid, garcinol, and curcumin are the most crucial HAT inhibitors obtained so far from plants. Anacardic acid and garcinol are the potent inhibitors of both p300 and PCAF, and their anti-cancer properties have also been studied: Anacardic acid has been shown to increase the sensitivity of human tumour cells to the cytotoxic effects of ionising radiation, providing a new treatment method for improving the efficacy of clinical radiotherapy. And among the four human leukaemia cell lines, Garcinol showed a significant growth inhibitory effect because of apoptosis mediated by caspase-3 activation.

Among these natural compounds, curcumin has been studied the most at present, and this compound has shown efficacy in preventing and treating various cancers. Several studies have shown that curcumin can affect many molecular and biochemical cascades, interact and regulate different targets, including transcription factors, growth factors and their receptors, as well as various protein kinases and metastases that cause malignant transformation. Studies have shown that curcumin is also a specific inhibitor of p300/CREB binding protein (CBP), which is a transcriptional co-activator that is frequently mutated and dysregulated in cancer [h]. Curcumin has also been shown to inhibit the expression of genes and pathways involved in cell apoptosis, cell invasion and adhesion. Because of the various mechanisms proposed, curcumin therapy has shown promise as a therapeutic drug. In fact, curcumin has been studied in human clinical trials for its effects on various tumours, including multiple myeloma, pancreatic cancer, myelodysplastic syndromes, and colon cancer. However, in these studies, the clinical effect is weak, which may be due to the poor bioavailability of the compound [15]. Compound based on the synthetic peptide CoA are the first class of potent and selective HAT inhibitors described for p300 and PCAF acetyltransferase. Coenzyme A (lys-CoA) binds to lysine specifically inhibits p300, while the long peptide of 20 amino acids (H3-CoA-20) targets PCAF. Unfortunately, these synthetic inhibitors have shown cell permeability problems and high metabolic instability, limiting their use in clinical and in vivo [16].

As mentioned briefly above, HDAC has been found to be associated with a large number of transcription and chromatin-related factors. In some cases, they are present in a multi-protein complex, including various HDACs and other enzymes. For example, DNMT1 and HDAC1 interact and form a complex that contains the transcription factor E2F and the tumour suppressor Rb [16]. Since HDAC has no DNA-binding activity, the interaction with complexes containing sequence-specific DNA-binding proteins promotes the acquisition of DNA and the recruitment of specific chromosomal regions. These co-inhibitory complexes (including YY1, Sin3A, N-CoR, and SMRT) direct HDAC activity to specific promoters to regulate transcription [17].

For HAT, the activities of different HDACs are not limited to targeting histones and non-histone proteins, including transcription factors and many others involved in DNA repair and replication, metabolism, cytoskeletal dynamics, apoptosis, nuclear import, protein folding and cellular signaling [18]. Therefore, HDACs promote the proper coordination of gene expression by regulating the acetylation of chromatin levels and control many aspects of biological processes, such as protein stability, protein translocation, enzyme activity, protein-protein interaction, and DNA binding affinity through acetylation of non-histone proteins. Role of HDACs in cancer is not limited to their contribution to histone deacetylation but also their role in non-histone deacetylation. In fact, many proteins that are involved in tumorigenesis, such as p53, HSP90, E2F, pRB, and BCL6, have been identified as substrates of HDAC [18]. Thus, inhibitors against HDAC have been extensively researched and developed because of their essential role in tumour development. Main effect of interference with HDAC activity is to cause the re-expression of genes with tumour suppressor function, thereby reversing the abnormal epigenetic state of tumour cells, cell cycle arrest, and induction of differentiation or apoptosis. Moreover, multiple synthetic or natural HDACi has shown significant antitumour effect in vivo whether used as a single agent or in combination therapy.

4. Long non-coding RNA

Long non-coding RNAs (lncRNA) are RNA types defined as transcripts with lengths exceeding 200 nucleotides that are not translated into protein. Non-coding RNA (ncRNA) provides another level of epigenetic regulation. Unlike what people usually think, most human transcripts do not encode proteins, but they play an essential role in cell differentiation and function. Regulatory ncRNAs can be divided into two subgroups based on their size: short-chain ncRNAs (including miRNA, siRNA, and PIWI-interacting RNA (piRNA)) and lncRNA. Recent studies have shown that ncRNAs, more specifically miRNAs and siRNAs, can effectively silence genes by changing histone deacetylation, methylation, and DNA methylation [9].

5. The different types of cell therapy

Chimeric antigen receptor (CAR) is a modular fusion protein that can be divided into extracellular target binding domain, which is usually derived from the single-chain variable fragment (scFv) of antibody, spacer domain, transmembrane domain, and intracellular signaling domain containing CD3z linked with zero or one or two costimulatory molecules such as CD28, CD137, and CD134 [1]. The incorporation of costimulatory molecules or cytokines can enable engineered T cells to destroy tumour cells effectively. CARs are produced by fusing the antigen-binding region of monoclonal antibodies or other ligands with transmembrane and intracellular signaling domains. They have recently shown clinical benefit in patients receiving CD19-directed autologous T cell therapy [19].

Most CAR-T cell trials have used autologous T cells for transduction. A cancer patient's T cells are collected, activated with antibodies or antibody-coated beads, and then transduced, most commonly with a lentivirus or retrovirus, to express the CAR molecule. CAR-T cells are then expanded in vitro to sufficient numbers to infuse back into the patient. However, CAR-T cells can also be derived from T cells of another healthy donor, which is allogeneic. Although CAR-T cell therapy has shown impressive outcomes in the clinical study, the challenges still need to be overcome.

The most noticeable side effect is cytokine release syndrome, a situation in which the immune system of patients was activated and releasing an increased number of inflammatory cytokines. CRS occurs in almost all patients treated with CAR-T cell therapy; in fact, the presence of CRS is a diagnostic marker that indicates the CAR-T cells are working as intended to kill the cancer cells [20]. These reactions indicate the strong interaction between CAR-T cells and cancer cells and host immune system cells, leading to CAR-T cell activation and expansion, and in some patients, cross-activation of immune cells and systemic cytokine activation. Vicious circle release can even reach toxic levels [21]. In order to achieve clinical efficacy while avoiding systemic cytokine toxicity, CAR T cells must reach the threshold level of activation and cytokine secretion without exceeding the level that leads to a vicious cycle of cytokine release. The degree and kinetics of CAR T cell activation are affected by the overall tumour burden, antigen expression level on tumour cells, and the affinity of scFv (or other antigen-binding domains) to the antigen, and the costimulatory elements included in CAR, as well as other factors. Neurological toxicity is also another side effect that is often associated with CAR-T cell treatment [22].

PD-1 is a type I transmembrane receptor that modulates the activity of T cells in peripheral tissues [23]. An activated T cell expresses PD-1 on its surface upon antigen recognition and produces interferons that induce expression of PD-L1 in multiple tissues. Binding of PD-1 to its ligand limits T-cell activity. Thereby, under normal conditions, the PD-1/PD-L1 pathway prevents excessive stimulation and maintains the immune tolerance to self-antigens by negatively regulating the immune response. PD-1 expression is induced upon the activation of CD4+ T cells, CD8+ T cells, NKT cells, B cells, and monocytes, whereupon it binds two distinct ligands, PD-L1 (B7-H1 or CD274) and PD-L2 (B7-DC. PD-L1 is constitutively and inducibly expressed by T and B cells, dendritic cells (DCs), macrophages, mesenchymal stem cells, bone marrow-derived mast cells, and on nonhematopoietic cells; PD-L2 expression is up-regulated on DCs, macrophages, and mast cells [24]. PD-1 is a monomeric type I surface glycoprotein consisting of a single V-set immunoglobulin superfamily (IgSF) domain attached to a transmembrane domain and a cytoplasmic domain with two tyrosine-based signaling motifs. PD-1 is often assigned to the CD28 receptor family, primarily based on functional similarities. However, PD-1 shares more structural homology with antigen receptors and CD8 and can be considered intermediate between the antigen receptors and CD28 family proteins, suggesting that a PD-1-like protein was a precursor of IgSF family signaling receptors. The specific protein's structure includes an extracellular IgV domain followed by a transmembrane region and an intracellular tail. The intracellular tail contains two phosphorylation sites in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, suggesting that PD-1 negatively regulates T-cell receptor TCR signals. This is consistent with the binding of SHP-1 and SHP-2 phosphatases to the cytoplasmic tail of PD-1 upon ligand binding. In addition, PD-1 ligation up-regulates E3-ubiquitin ligases CBL-b and c-CBL that trigger T cell receptor down-modulation [25].

CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), also known as CD152 (cluster of differentiation 152), is a protein receptor that functions as an immune checkpoint and downregulates immune responses. CTLA4 is homologous to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 and CD86, also called B7-1 and B7-2, respectively, on antigen-presenting cells. CTLA-4 binds CD80 and CD86 with greater affinity and avidity than CD28, thus outcompeting CD28 for its ligands. The protein contains an extracellular V domain, a transmembrane domain, and a cytoplasmic tail. Alternate splice variants encoding different isoforms have been characterized. The membrane-bound isoform functions as a homodimer interconnected by a disulfide bond, while the soluble isoform functions as a monomer. The intracellular domain is similar to that of CD28 in that it has no intrinsic catalytic activity and contains one YVKM motif able to bind PI3K, PP2A and SHP-2 and one proline-rich motif able to bind SH3 containing proteins. The first role of CTLA-4 in inhibiting T cell responses seem to be direct via SHP-2 and PP2A dephosphorylation of TCR-proximal signaling proteins such as CD3 and LAT. CTLA-4 can also affect signaling indirectly via competing with CD28 for CD80/86 binding. CTLA-4 can also bind PI3K, although the importance and results of this interaction are uncertain.

However, PD-L1 is often overexpressed in different tumours, including lymphoma, melanoma, lung, breast cancer, glioblastoma, ovarian, kidney tumours, and bladder cancers, which results in immune response handicap within the tumour microenvironment. The PD-1/PD-L1 interaction inhibits T-lymphocyte proliferation, the release of cytokines, and cytotoxicity, resulting in exhaustion and apoptosis of tumour-specific T cells. Blockage of the PD-1/PD-L1 interaction results in reversal of exhausted T-cell phenotype and normalization of antitumor response, providing the rationale of targeted therapy.

6. Approved checkpoint inhibitors

The first immune checkpoint inhibitor (ipilimumab as an anti-CTLA-4 antibody) was approved by the FDA as for the treatment of melanoma that crated a footstep in immunotherapy cancer treatment. Currently, the two classes of immunotherapy that have been FDA approved for clinical use are (1) inhibitors of either the programmed death receptor 1 (PD-1) or its ligand (PD-L1), or (2) cytotoxic T-cell lymphocyte-associated protein 4 (CTLA-4).

Cancer cells produce antigens, and the immune system can use these antigens to recognize them. These antigens are recognized by dendritic cells, after which the dendritic cells transmit the antigen information to the cytotoxic T lymphocytes (CTL) in the lymph nodes. CTLs recognize those cancer cells through antigens provided by dendritic cells and destroy them. However, along with the antigen, dendritic cells transmit inhibitory signals. This signal binds to the receptor cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) on the CTL and then shuts down the cytotoxic response. This mechanism allows cancer cells to escape the immune system [26].

Ipilimumab can bind to CTLA-4 to block the inhibitory signal and allow CTL to destroy cancer cells again. In addition, a 2014 study showed that the specific role of the antibody is to allow patients' T cells to target a wider variety of antigens rather than increasing the number of attacks against a single antigen.

Nivolumab is a genetically engineered anti-PD-1 mAb, developed by immunizing transgenic mice for human immunoglobulin loci with recombinant Chinese hamster ovary cells expressing human PD-1 and PD-1/human IgG1 Fc fusion protein. Nivolumab contains a hinge region mutation (S228P), the S228P mutation reduces Fc exchange with serum IgG4 molecules to improve stability and reduce therapeutic variability. In addition, Nivolumab binds PD-1 with high affinity (KD=2.6 nmol/L by Scatchard analysis to polyclonally activated human T cells), blocking its interactions with PD-L1 and PD-L2, and stimulates memory response to tumour antigen-specific T cell proliferation [27].

The PD-1 and PD-L1 is a receptor-ligand system, and in tumour microenvironment they are attached to each other, resulting blockade of antitumor immune responses. PD-1 is mainly expressed on the immune system's T cells, whereas PD-L1 is on the cancer cells and antigen-presenting cells. Therefore, the inhibitors that block the interaction of PD-1 and PD-L1 will cause the resurrection of the T-cell mediated antitumor immune effect. The PD-1 and PD-L1 antibody inhibitors have been designed to block either the PD-1 or the PD-L1 side and turn on T-cell mediated immunity. Currently, it is not clear whether the PD-1 and PD-L1 inhibitors are more effective. The effectiveness of PD-1 and PD-L1 inhibitors depends on patients' characteristics, such as (i) gender, (ii) types of tumours, (iii) mutation, translocation of genes (EGFR, Kras, ALK), and (iv) metastases of tumour [28]. As the tumour is heterogeneous in nature, the expression of PD-L1 is not uniform, thus PD-L1 immunohistochemistry staining varies with tumour locations. Therefore, the indication of PD-L1 expression and response of PD-L1 inhibitors remain debatable and needs to be understood deeply.

7. Improving the efficiency of car-t cells

In order to improve the efficiency of CAR-T cells, a significant challenge is countering the antigen escape effect. Despite high initial response rates, antigen escape — that is, complete or partial loss of target antigen expression by the cancer cells — is observed in a notable proportion of patients treated with CAR T cells. Therefore, using different CAR-T cell products that target alternative antigens for

the sequential treatment of patients has been clinically useful [29]. Although the option that engineers a single CAR-T cell product to let it have multiple specificities against several targets is an attractive way, multi-target CAR-T cells can be created by mixing different CAR-T cell products that target a single antigen before the infusion or by transducing T cells with multiple CAR constructs [30]. Alternatively, bi-specific CAR-T cells can be created by engineering a single CAR molecule with two distinct binding domains [30].

Another multi-targeted strategy involves further modification of CAR T cells to secrete bi-specific T cell engagers (BiTEs). BiTE usually consists of two scFvs, one for CD3 and the other for TAA, connected through a flexible linker; therefore, these drugs can physically connect T cells to cancer cells. It is worth noting that the BiTE drug blinatumomab, which targets CD19, has been approved by the FDA for the treatment of ALL [31].Concerning prolonging the persistence of CAR T cells in patients, one of the most promising strategies currently seems to be to use T cell populations with a higher percentage of less differentiated T cell subsets with higher proliferation capacity, such as naive T cells, stem cell memory T (TSCM) cells and central memory T (TCM) cells [32].

References

[1] Hood, E. RNAi: What's all the noise about gene silencing? Environmental Health Perspectives 112, A224–A229 (2004).

[2] Mocellin, S. & Provenzano, M. RNA interference: learning gene knockdown from cell physiology. Journal of Translational Medicine 2, 39 (2004).

[3] Bird, A. DNA methylation patterns and epigenetic memory. Genes Dev. 16, 6–21 (2002).

[4] The fundamental role of epigenetic events in cancer | Nature Reviews Genetics.

[5] Baylin, S. B. DNA methylation and gene silencing in cancer. Nat Rev Clin Oncol 2, S4–S11 (2005).

[6] Bestor, T. H. The DNA methyltransferases of mammals. Human Molecular Genetics 9, 2395–2402 (2000).

[7] Knudson, A. G. Two genetic hits (more or less) to cancer. Nat Rev Cancer 1, 157–162 (2001).

[8] Galm, O., Herman, J. G. & Baylin, S. B. The fundamental role of epigenetics in hematopoietic malignancies. Blood Reviews 20, 1–13 (2006).

[9] Novel classes of non-coding RNAs and cancer | SpringerLink.

[10] Silverman, L. R. Targeting Hypomethylation of DNA to Achieve Cellular Differentiation in Myelodysplastic Syndromes (MDS). The Oncologist 6, 8–14 (2001).

[11] Aparicio, A. & Weber, J. S. Review of the clinical experience with 5-azacytidine and 5-aza-2'-deoxycytidine in solid tumors. Curr Opin Investig Drugs 3, 627–633 (2002).

[12] Finch, J. T. et al. Structure of nucleosome core particles of chromatin. Nature 269, 29–36 (1977).

[13] Sarma, K. & Reinberg, D. Histone variants meet their match. Nat Rev Mol Cell Biol 6, 139–149 (2005).

[14] Histone Modification Therapy of Cancer - ScienceDirect.

[15] Bar-Sela, G., Epelbaum, R. & Schaffer, and M. Curcumin as an Anti-Cancer Agent: Review of the Gap between Basic and Clinical Applications. Current Medicinal Chemistry 17, 190–197 (2010).

[16] HATs off: Selective Synthetic Inhibitors of the Histone Acetyltransferases p300 and PCAF - ScienceDirect.

[17] Yang, X.-J. & Seto, E. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. Nat Rev Mol Cell Biol 9, 206–218 (2008).

[18] Glozak, M. A., Sengupta, N., Zhang, X. & Seto, E. Acetylation and deacetylation of non-histone proteins. Gene 363, 15–23 (2005).

[19] Dai, H., Wang, Y., Lu, X. & Han, W. Chimeric Antigen Receptors Modified T-Cells for Cancer Therapy. JNCI: Journal of the National Cancer Institute 108, (2016).

[20] Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ (2016). "Toxicity and management in CAR-T cell therapy". Molecular Therapy Oncolytics.

[21] Brudno, J. N. & Kochenderfer, J. N. Recent advances in CAR T-cell toxicity: mechanisms, manifestations and management. Blood Rev. 34, 45–55 (2019).

[22] Brudno, J. N. & Kochenderfer, J. N. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood 127, 3321–3330 (2016).

[23] Ishida Y, Agata Y, Shibahara K, Honjo T (November 1992). "Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death". The EMBO Journal. 11 (11): 3887–95.

[24] Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, Yagita H, Honjo T (May 1996). "Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes". International Immunology. 8 (5): 765–72.

[25] Characterization of CTLA-4 structure and expression on human T cells.

[26] Komohara, Y. et al. Anti-Cancer Immune Reaction and Lymph Node Macrophage; A Review from Human and Animal Studies. Immuno 1, 223–230 (2021).

[27] RF, F. et al. Nivolumab-Induced Rapid Tumor Remission of Pulmonary Adenocarcinoma. Oncology & Cancer Case Reports 03, (2017).

[28] D' Incecco, A. et al. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. Br J Cancer 112, 95–102 (2015).

[29] Pan, J. et al. CD22 CAR T-cell therapy in refractory or relapsed B acute lymphoblastic leukemia. Leukemia 33, 2854–2866 (2019).

[30] Bailey, S. R. & Maus, M. V. Gene editing for immune cell therapies. Nat. Biotechnol (2019).

[31] Kantarjian, H. et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. N. Engl. J. Med. 376, 836–847 (2017).

[32] Sadelain, M., Riviere, I. & Riddell, S. Therapeutic T cell engineering. Nature 545, 423–431 (2017).