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Review

Strategies to overcome low MHC-I expression in paediatric and adult tumours

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Summary

Immunotherapy has made significant advancements in cancer treatments, improving patients' survival rates and quality of life. Several challenges still need to be addressed, which include the considerable fraction of incomplete curative responses in cancer patients, the development of therapy resistance by tumours, and the occurrence of adverse effects, such as inflammatory and autoimmune complications. Paediatric tumours usually exhibit lower responsiveness to immunotherapies compared to adult tumours. Although the underlying reasons are not yet fully understood, one known mechanism by which tumours avoid immune recognition is through reduced cell surface expression of major histocompatibility complex class I (MHC-I) complexes. Accordingly, the reduced presentation of neoantigens by MHC-I hinders the recognition and targeting of tumour cells by CD8+ T cells, impeding T-cell-mediated cytotoxic anti-tumour responses. MHC-I downregulation indeed often correlates with a poorer prognosis and diminished response to immunotherapy. Understanding the mechanisms underlying MHC-I downregulation in different types of paediatric and adult tumours is crucial for developing strategies to restore MHC-I expression and enhance anti-tumour immune responses. We here discuss progress in MHC-I-based immunotherapies against cancers.

Keywords: MHC-I expression, MHC-I downregulation, pediatric oncology, immunotherapy, immune evasion

Abbreviation: MHC-I: Major histocompatibility complex class I; NSCL: Non-small cell lung cancer; NB: Neuroblastoma; TMB: Tumour mutational burden; HLA-I: Human leukocyte antigen class I; NGS: Next-generation sequencing; ESC: Embryonic stem cell; pMHC-I: Peptide:MHC-I; TCR: T cell receptor; NK: Natural killer; KIR: Killer cell immunoglobulin-like receptor; IRF1: Interferon regulatory factor 1; miR: MicroRNA; GSL: Glycosphingolipid; LOH: Loss of heterozygosity; CTLA-4: Cytotoxic T-lymphocyte antigen; PD-1: Programmed cell death 1; TIL: Tumour-infiltrating T lymphocyte; IFN: Interferon; DUX4: Double homeobox 4; LNK: Lymphocytes adapter protein; N4BP1: Nedd4 Binding Protein; TNIP1: TNF-alpha-induced protein 3 interacting protein 1; DNMT: DNA methyltransferase inhibitor; HDAC: Histone deacetylase; RA: Retinoic acid.

Introduction

Each year, approximately 400 000 children and adolescents aged 0–19 years are diagnosed with cancer [1]. Immunotherapies, including immune checkpoint blockade, antibody-mediated therapy, and therapeutic cancer vaccines, demonstrated remarkable success in various adult cancers, including melanoma and non-small cell lung cancer (NSCL) [2, 3]. Compared with conventional treatments such as chemotherapy, radiotherapy, and surgery, immunotherapy has significantly improved survival rates and quality of life for patients [4, 5]. Not all patients, however, respond to immunotherapy or show partial reactivity, and some patients experience relapse after an initial response [6, 7]. Furthermore, paediatric tumours, such as neuroblastoma (NB), have shown limited responsiveness to immunotherapies [8]. The underlying reasons for this disparity are not yet fully understood.

A high tumour mutational burden (TMB), reflective of the number of DNA mutations in a tumour, has been denoted as a crucial predictor of response to immunotherapy [9, 10]. When leading to alterations in protein structure and composition, DNA mutations can result in the generation and possibly presentation of alternative antigens known as neoantigens. Malignant cells can express neoantigens via major histocompatibility complex I (MHC-I) molecules. Consecutively, CD8+ T cells, through their T-cell receptors, recognize specific neoantigen peptide/MHC-I complexes, which trigger a cytotoxic immune response against cancer cells presenting specific neoantigen-derived peptides [11]. Interestingly, one major mechanism through which many tumours may avoid anti-tumour immunity is the downregulation of MHC-I, which causes reduced recognition by- and cytotoxicity of CD8+ T cells [12, 13]. Despite that MHC-I downregulation poses a huge challenge for current T-cell engaging immunotherapies, the understanding of the nature of dysregulation provides an opportunity to restore MHC-I expression in adult tumours. In contrast, our understanding of peptide/MHC-I complex formation and regulation and the neoantigen landscape in paediatric tumours remains incomplete. There are notable differences in cancer genetics, distribution and the microenvironment between adult and paediatric cancers [14]. Adult-type cancers usually develop by malignant conversion of end-differentiated cells. Paediatric cancers instead

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more usually arise from undifferentiated embryonic tissues that inherently lack immunogenicity, including receptors and regulators involved in immunological responses [15]. Paediatric tumours are characterized by reduced expression of MHC-I, with a low TMB and a correspondingly low neoantigen load [9, 16]. Consequently, distinct therapeutic approaches must be developed when one aims to increase MHC-I expression on tumour cells, comparing cancers from adult-type with those of paediatric origins. Upregulating MHC-I expression in various cancer types holds promise as an immunotherapy strategy, that is to (re)activate immune control of tumours. It has the potential to improve response rates to immunotherapies and enhance efficacy against previously resistant cancers [17]. Therapeutic strategies that aim to restore MHC-I expression are currently being investigated in adult tumours, and involve the targeting of cell biological mechanisms that mediate MHC-I depletion [18, 19]. This review provides an overview of reduced MHC-I expression in relation to cancer development and its implications for immunotherapy efficacy, highlighting the differences between adult and paediatric tumours. Furthermore, we discuss potential therapeutic strategies to restore MHC-I expression and overcome immunotherapy resistance in paediatric tumours.

MHC-I expression and its role in anti-tumour immunity

Expression and function of MHC-I molecules in the body

The human leukocyte antigen class I (HLA-I) is the human equivalent of MHC-I and is highly polymorphic. HLA-I comprises three classical loci (HLA-A, -B, and -C) and three non-classical loci (HLA-E, -F, and -G) [11]. Its expression is highly regulated. For example, different tissues have extraordinary heterogeneity in classical HLA-I expression levels, as was shown using next-generation sequencing (NGS) and RNA sequencing data from human non-cancer tissues, which allowed for mapping HLA class I distribution throughout the body [20]. Classical HLA-I molecules are highly expressed in lymphatic tissues, lymph nodes, and spleen, mainly attributed to the increased presence of antigen-presenting cells, while the lowest classical HLA-I expression is found in privileged organs such as the brain, retina, testis, and muscle [21]. Furthermore, studies indicate that the expression of MHC-I varies during cell development [22, 23]. For instance, MHC-I expression in human embryonic stem cells (ESC) is initially low but can be swiftly induced upon cellular differentiation [24]. The structure of MHC-I molecules and the antigen presentation pathway have been expertly reviewed elsewhere [25, 26], and are summarised in Fig. 1.

The immune system can recognize malignantly transformed cells, such as through the expression of neoantigens loaded into MHC-I molecules that distinguish them from non-transformed cells. MHC-I molecules play a key role in the interaction between T cells of the adaptive immune system and malignant cells [27]. In brief, the T cell receptor (TCR) of CD8+ T cells can specifically recognise an antigen in the form of a short peptide that is bound by MHC-I molecules on the surface of target cells [28]. This triggers a plethora of different effector functions in CD8+ T cells, including the ability to eliminate cancer cells via cytokine/granzyme secretion, perforin, or FAS-dependent pathways [29, 30] (Fig. 1). In contrast, natural killer (NK) cells are inhibited by MHC-I via inhibitory receptors such as killer cell immunoglobulin-like receptors (KIRs) [31]. Therefore, tumours with low expression of MHC-I might be more susceptible to NK-cell-induced cytotoxicity, as proposed as the missing-self hypothesis by Kärre *et al.* [32]. The underlying mechanisms of MHC-I interaction with either CD8+ T cells and NK cells have been assessed in excellent reviews elsewhere [33–35].l

MHC-I expression is crucial in the adaptive immune response, although it is not essential for cell viability or growth. As stated, tumours may employ MHC-I downregulation as a primary mechanism to evade recognition by the adaptive immune system [36], as is seen in a broad variation of tumour types including melanoma, breast, colorectal, and NSCL carcinomas [37–40]. While there has been extensive research on MHC-I expression in adult cancers, studies on paediatric cancers are relatively limited. This is accompanied with a low annual number of patients compared to adult cancers. It is important to recognize that paediatric tumours should be considered a distinct subset separate from adult tumours and they present unique therapeutic challenges mostly due to large differences in development, cancer genetics, distribution, and tumour microenvironment [14].

Differences between adult and paediatric tumours

Adult tumours typically arise from the accumulation of DNA mutations over time, with more malignancies occurring in individuals with advanced age [41]. Unhealthy lifestyle factors including smoking, alcohol consumption, and UV exposure are known triggers for such mutations and subsequently tumourigenesis [42, 43]. However, the same paradigm cannot be applied to paediatric tumours, which more often originate from undifferentiated stem or progenitor cells that undergo oncogenic mutations during specific developmental stages [44–46]. It is likely that these cells possess a distinct transcriptional program, influenced by genetic or epigenetic alterations, that promotes tumour development. Paediatric tumours often do not harbour MHC-I expression in the first place, as evidenced by various studies (see Tables 1 and 2). Since most paediatric tumours harbour a significantly lower presence of mutations compared to adult tumours, paediatric tumours often lack identifiable targets to develop specific and effective therapies.

MHC-I expression and downregulation during tumour development

The infiltration of specific antitumour CD8+ T cells into the tumour tissue in the course of normal immune defence results in their recognition and elimination of MHC-I positive cells, which in a tug-of-war type of response can induce in tumour cells an overall selection process that benefits MHC-I negative cells [75]. Eventually, tumours can become completely MHC-I negative and acquire an encapsulated structure that is ‘non-permissive’ for immune attack [58]. This phenotype is often associated with metastasis, although the MHC-I expression phenotype in metastatic tumours can exhibit considerable diversity and may not always involve a total loss of MHC-I expression [76] (Figure 2).

Genetic defects that affect MHC-I presentation in tumours include point mutations, base pair insertion, or deletion, which are irreversible processes. Examples include genetic mutations and deletions in the MHC-I heavy chain or β2M genes, or

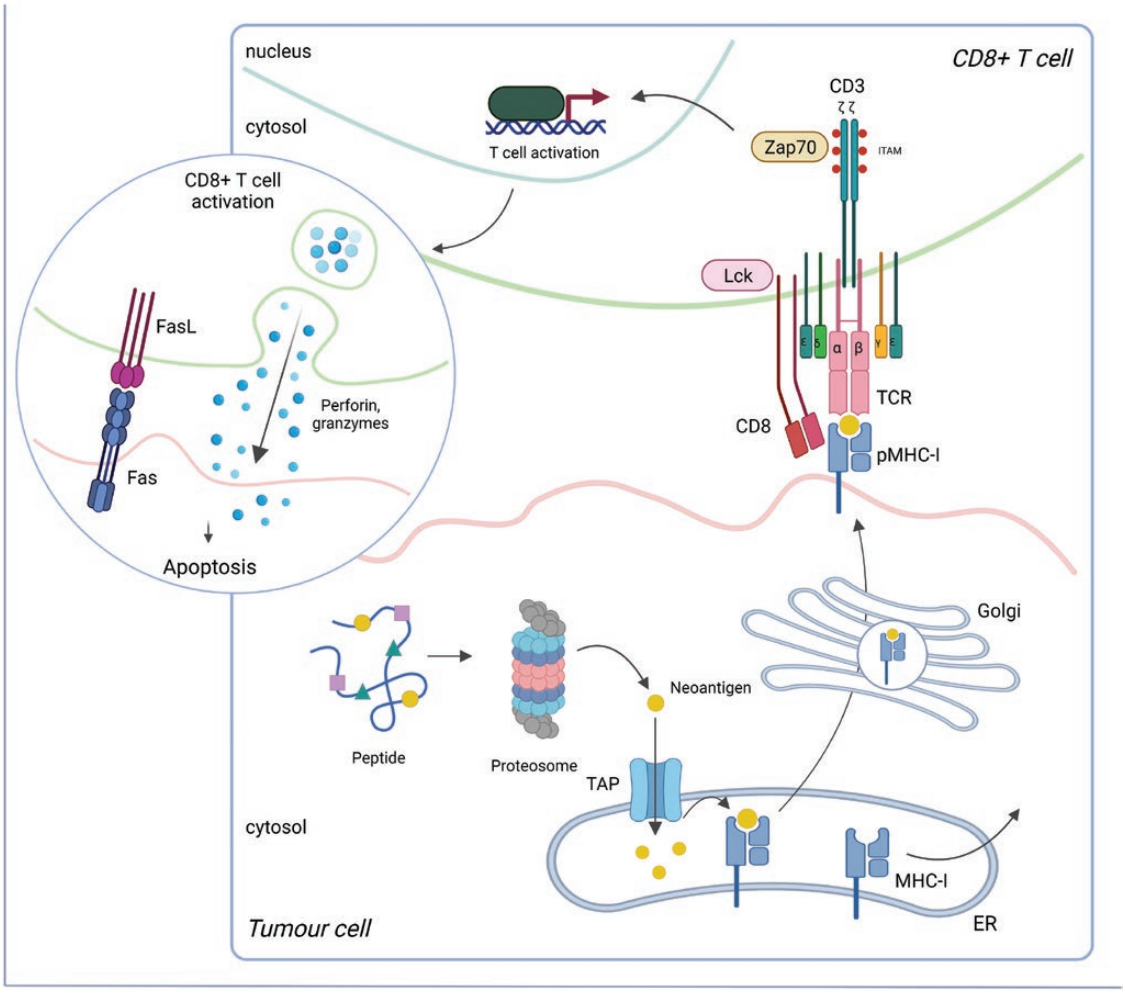

Figure 1. Antigen processing and presenting pathway. Proteins are degraded into peptides by the proteosome and transported from the cytosol into the ER lumen by TAP transporter. In the ER, MHC-I molecules are folded, which allows them to bind to specific peptides, called antigens. The resulting peptide:MHC-I (pMHC-I) complex is transported to the plasma membrane through the Golgi apparatus. Then, CD8+ T cells recognize and bind the pMHC-I complex via their TCR receptor and their co-receptor CD8. The TCR complex is formed; it consists in the TCRα-β subunits and three CD3 co-receptor signalling subunits (γ, ε, δ). CD8 recruits Lck, that phosphorylates ITAM motifs on the intracellular part of CD3. Next, Zap70 binds to the complex and causes the activation of various downstream pathways leading to the activation of the cell. CD8+ T cells induce apoptosis in the tumour cells, secrete perforin, and granzymes, and upregulate the expression of Fas ligand on the cell surface (created with Biorender.com).

Table 1. Examples of adult tumours with changed MHC-I expression relative to healthy cells

Tumour type	MHC-I expression	Prognostic indication	References
Melanoma	Downregulated	Association with immunotherapy resistance and T cells infiltration	Lim *et al.* [38] Lee *et al.* [47] Shklovskaya *et al.* [48]
NSCL	Loss or downregulated	Association with poor survival and ICIs outcome	Montesion *et al.* [39] Hurkmans et al. [49] Ichinokawa *et al.* [50]
Colorectal cancer	Loss or downregulated	Worst CD8+ T cells activation and response	Zhang *et al.* [51] Geng *et al.* [52] Moretti *et al.* [53]
Breast cancer	Loss or downregulated	Poor prognosis and response	Fang *et al.* [54] Dusenbery *et al.* [37] Zhao *et al.* [55]
Prostate cancer	Downregulated		Kowarschik *et al.* [56] Korentzelos *et al.* [57] Garrido *et al.* [58]

Table 2. Examples of paediatric tumours with changed MHC-I expression relative to healthy cells

Tumour type	MHC-I expression	Prognostic indication	References
Neuroblastoma	Low or absent	Limitation of cytotoxic T-cell engagement, function-nal alteration of the TME	Cornel *et al.* [59] Lorenzi *et al.* [60] Pistoia *et al.* [61] Wölfl *et al.* [62]
Medulloblastoma	Low or absent	Poor prognosis	Garancher *et al.*[63] Smith *et al.* [64] Smith *et al.* [65]
Ewing sarcoma	Low	Poor prognosis	Biele *et al.* [66] Peters *et al.* [67] Thiel *et al.* [68]
ALL and AML	Expressed or downregulated	Downregulation of MHC-I is infrequent but functionally relevant	Chen *et al.* [69] Depreter *et al.* [70] Kang *et al.* [71]
Osteosarcoma	Downregulated, variable expression	Association with immuno-suppressive TME	Liu *et al.* [72] Tian *et al.* [73] Delgado *et al.* [74]

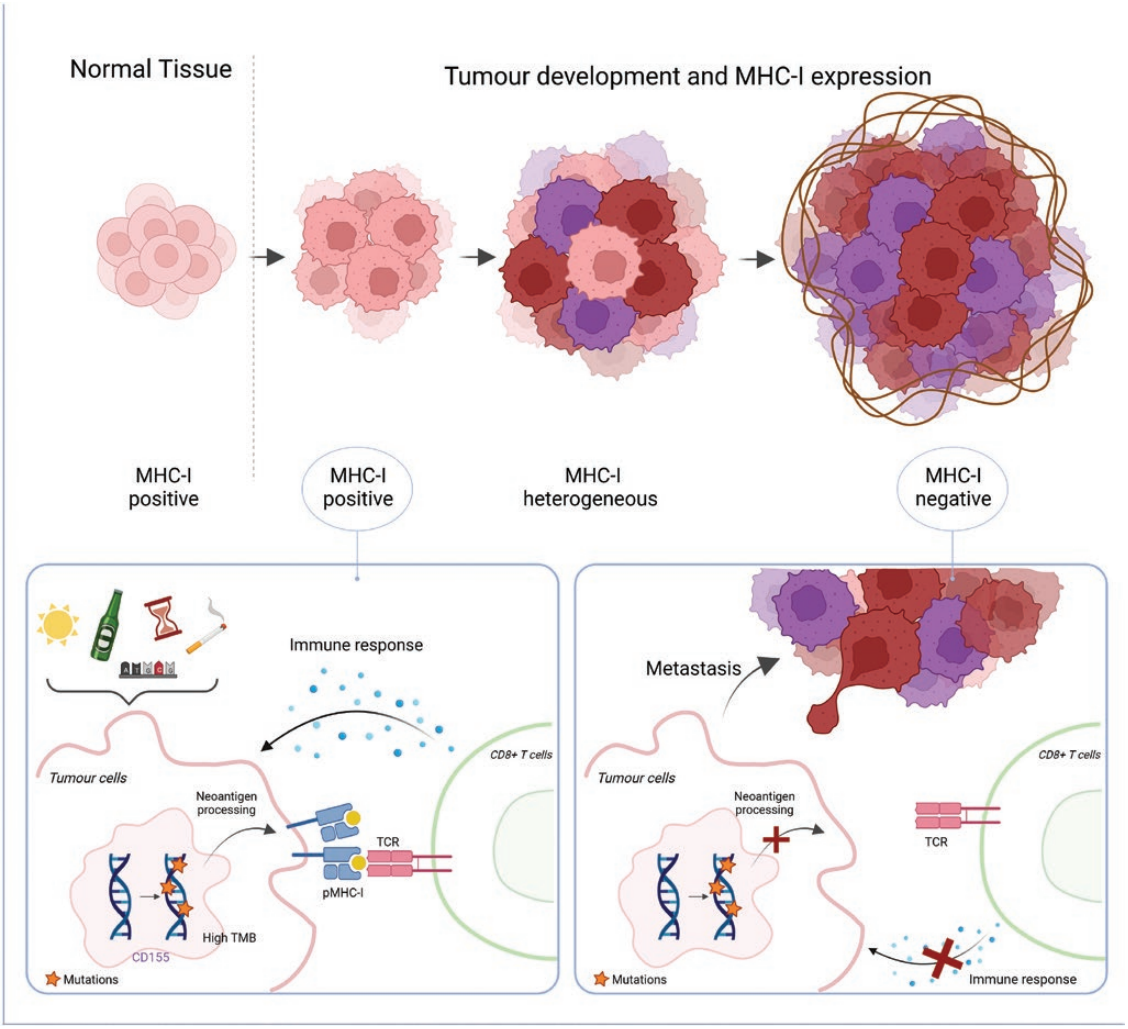

Figure 2. MHC-I expression and downregulation during tumour development. Tumour cells are normally MHC-I positive at early stage of the tumour development. Mutations, caused by environmental or genetic factors, are translated in neoantigens, which can be presented on the cell surface via MHC-I. CD8+ T cells recognize pMHC-I and trigger a cytotoxic immune response against tumour cells that present the neoantigens. The immunity selection causes heterogeneity in MHC-I expression amongst tumour cells. Finally, tumours can be completely MHC-I negative and the CD8+ T cells response is repressed. MHC-I negative tumours lead to the formation of metastasis (created with BioRender.com).

in the TAP or Tapasin genes, that lead to downregulation or complete loss of MHC-I [77]. Indeed, a study showed that restoring the expression of TAP-1/TAP-2 in murine melanoma knockout models enhances cell-surface expression of MHC-I and significantly reduces subcutaneous tumour growth [78]. In addition, mutations in the β2M gene correlate with the absence of MHC-I in metastatic melanoma patients [79]. Tumour cells can also contain reversible defects that affect MHC-I expression. MHC-I downregulation in cancer can also be attributed to epigenetic and/or (post)-transcriptional dysregulation of pathways involved in the transcription of MHC-I heavy chain, β2M genes, or components of the antigen processing and presentation machinery. Transcription factors such as interferon regulatory factor 1 (IRF1), NF-kB, and NLRC5 play crucial roles in regulating MHC-I gene expression. Reduced expression of NF-kB and IRF1 in certain aggressive NB cell lines correlates with significantly decreased expression of MHC-I [60]. Furthermore, truncating/splice mutations or missense mutations in NLRC5 are associated with poor prognosis, low MHC-I expression, and diminished levels of infiltrating immune cells in melanoma [80]. In addition, loss of MHC-I in cancer can result from post-transcriptional regulation of mRNA, mediated by non-coding RNAs. MicroRNAs (miRs) are key players in this process, impacting various components of the antigen processing and presentation machinery. For instance, the overexpression of two specific miRs in melanoma cells is found to downregulate the TAP1 protein, thereby reducing the expression of MHC-I cell-surface antigens [81]. Moreover, alterations in several oncogenic pathways, including MAPK, epidermal growth factor receptor, HER2, and c-MYC pathways, can also influence MHC-I expression in various cancers [12].

In cancer cells, MHC-I expression can be modulated by the perturbation of trafficking and degradation of MHC-I molecules. At the plasma membrane, MHC-I molecules can diffuse laterally, which provides the option to interact with other cell-surface molecules [82]. For example, MHC-I molecules were shown to interact with glycosphingolipids (GSLs), hydrophobic ceramide units decorated with variable hydrophilic sugar structures [83]. Overexpression of GSLs has been observed in several tumour types including glioma, AML, and adenocarcinomas [84–86]. High levels of GSLs interfere with the accessibility of T cells and immune cell receptors to bind tumour-expressed molecules including MHC-I [83]. Another way through which tumour cells regulate MHC-I display and its accessibility on the cell surface is via MARCH proteins. MARCH9 and its homologue MARCH4 target and degrade MHC-I molecules in a ubiquitin-dependent manner [87]. Several tumours overexpress MARCH proteins which may support immune evasion by lowering the cell-surface display of MHC-I proteins [88]. Furthermore, MARCH9 is required at the trans-Golgi network to promote MHC-I endosomal recycling. The acid environment of the endosome promotes the release of the bound peptide allowing for new peptide loading to occur into MHC-I, after which the MHC-I molecule can be recycled to the cell surface [87]. However, tumour cells may eliminate this recycling option by decreasing the endosomal below pH 4.5 leading to full and definite dissociation and degradation of the MHC-I complex [89].

Correlation between MHC-I expression and TMB

The advent of high-throughput methods, most notably NGS, revolutionized the detection and quantification of acquired mutations at the individual cancer genome level. TMB serves as a quantifiable measure of the number of mutations present in a tumour. Theoretically, the higher the TMB, the greater the number of neoantigen peptide possibilities that are available for presentation via MHC-I molecules to CD8+ T cells [9]. At the time of writing this review, the correlation between the number of mutations and MHC-I phenotype is an active area of research.

Certain adult cancers, such as melanoma, exhibit a high TMB [90, 91]. The analysis of 9175 tumour samples showed that tumours with a low TMB often exhibit low MHC-I expression, and vice versa [92]. In contrast, paediatric tumours generally exhibit a low TMB. A comprehensive analysis encompassing 961 tumours from children, adolescents, and young adults showed that mutation frequencies in paediatric tumours were approximately 14 times lower than in adult cancers [93, 94]. The insufficient neoantigen density in malignancies with low TMB, such as AML and paediatric brain cancers, requires more powerful strategies for the accurate identification of immunogenic neoepitopes that can be presented via MHC-I to CD8+ T cells.

Furthermore, changes in neoantigen presentation can occur independently from MHC-I expression. In cancer, events such as alternative mRNA splicing, premature termination of translation, and protein misfolding often occur and all these are involved in antigen presentation [95]. For example, in leukemic cells, a naturally occurring antigen encoded by alternatively spliced TTK transcript has been identified. The isolated T cells specific for this antigen fail to recognize most leukemic cells expressing the alternative TTK transcript [96]. The so-called loss of heterozygosity (LOH), which is a loss of either the maternal or paternal HLA haplotype, reduces the capacity of MHC-I to present antigen. Indeed, it has been shown that patients with tumours that underwent LOH were less responsive to immunotherapy compared to patients with tumours that had intact alleles [97]. Furthermore, LOH in NSCLCs was associated with higher TMB and increased immune evasion [98]. Overall, these data suggest that not all neoantigens are necessarily immunogenic targets for antigen-specific CD8+ T cells, highlighting a potential role for NK-cell-based immunotherapies. Furthermore, the numerous differences observed between paediatric and adult tumours underscore the importance of considering paediatric cancer as a distinct entity when developing therapeutic strategies.

Implication in immunotherapy

The discovery of immune checkpoints, such as cytotoxic T-lymphocyte antigen (CTLA-4) and programmed cell death 1 (PD-1) has been crucial to the development of cancer immunotherapy. Despite the improved therapeutic prospects, treatment with immune checkpoint inhibitors (ICIs) still has considerable limitations and challenges. Although a significant fraction of patients respond to ICIs, a large percentage of patients experience intrinsic or acquired resistance, partially depending on the tumour type. Intrinsic resistance refers to patients who do not respond at all to ICIs, while acquired resistance alludes to patients who have a period of an initial response to ICI therapy followed by progression of the disease [99]. Acquired resistance to ICIs in many types of cancer is associated with alterations in the MHC-I-related pathways [38, 47]. The efficacy of these immunotherapies is mainly based on the (re)activation of CD8+ T cells. If MHC-I is not present or is downregulated, CD8+ T cells cannot get

(re)activated and this usually correlates with a poor response to immunotherapy.

MHC-I downregulation and immunotherapy response

MHC-I expression level can be associated with the efficacy and duration of immunotherapy treatments. In a study for the expression of MHC-I in 10 metastatic lesions obtained from a melanoma patient undergoing immunotherapy, eight metastases were regressing after immunotherapy while two were progressing. The eight regressing metastases showed high levels of MHC-I expression, whereas the two progressing lesions had low MHC-I levels [100]. Similar results were found in two other studies performed by the same group. Briefly, a quantitative score was developed to correlate the tumour cell expression of antigen-presenting MHC-I molecules with the response to PD-1 monotherapy. They found robust MHC-I expression in all patients who responded to the therapy [48]. Furthermore, they described MHC-I downregulation as a hallmark of resistance to PD-1 inhibitors in melanoma patients [47]. Overall, MHC-I expression seems to play a major role in the response to immunotherapy. Understanding how MHC-I is downregulated might be crucial to determine the mechanism underlying the acquired resistance to immunotherapies. Restoring MHC-I expression could be a promising solution to solve the problem of adaptive resistance.

How MHC-I downregulation can contribute to adaptive immunotherapy resistance

Tumours with a high TMB usually respond better to anti-CTLA-4 and anti-PD-1 therapies [91, 101–103]. Interestingly, mutations in the β2M gene or genes involved in the IFN-γ pathway are positively associated with resistance to PD-1 treatment [104]. Those mutations can be responsible for MHC-I expression downregulation. Moreover, several studies showed a direct correlation between MHC-I expression and the increased presence of tumour-infiltrating T lymphocytes (TILs) [47, 48, 51]. For instance, a high intratumoural infiltration of CD8+ TILs can be positively associated with high MHC-I levels and with the regression of melanoma lesions. In contrast, the absence of TILs is often accompanied with low MHC-I expression and progression of tumour lesions [105]. Furthermore, LOH of the HLA locus did lead to a higher resistance against TILs in a CRC tumour [97] while another study showed that LOH of the HLA locus is associated with increased PD-L1 expression [98]. TIL profiling is also another valuable method to predict immunotherapy outcomes. In a study where samples from 46 patients with metastatic melanoma were obtained before and during anti-PD-1 therapy, patients responding to the treatment showed proliferation of intratumoural CD8+ T cells, which directly correlated with a reduction in tumour size [106]. Interestingly, not only TIL presence but also TIL differentiation and localization have been shown to determine clinical outcomes [107]. As CD8+ TILs recruitment and activation depend on MHC-I expression, restoring MHC-I expression might increase the presence, proliferation, and activity of TILs inside the tumour, and lead to a higher response to immunotherapy.

MHC-I expression in paediatric tumours

Fewer children than adults with cancer benefit from treatment with ICIs. While many factors may be responsible, this difference likely is contributed by the low TMB as well as low MHC-I expression in the majority of paediatric tumours [108]. Preclinical data suggested CTLA-4 as a promising target in paediatric melanoma and other solid tumours because of its high expression by TILs [109]. However, a recent phase I study (NCT01445379) with paediatric cancer patients treated with CTLA-4 blockade (Ipilimumab) revealed many side effects after patients were administered a single dose and did not show an anti-tumoural response [110]. Similar results were obtained in a phase II study of PD-1 antibody in children affected by bone sarcomas. They found similar side effects to the adult studies, but no antitumour effects (NCT02301039) [111]. Furthermore, in the clinical trial KEYNOTE-051 (NCT02332668), a paediatric study including patients affected by melanoma, lymphoma, solid tumour, and classical Hodgkin lymphoma, demonstrated tolerance to PD-1 therapy at adult doses, and again no responses towards tumour regression [112]. Overall, checkpoint inhibition-based immunotherapy has so far not proven overly successful in paediatric clinical trials [113].

Rescue MHC-I low phenotype

The reduction of MHC-I on selected tumour cell types eliminates the inhibitory signals initiated by MHC-I, leading to enhanced NK cell activation and increased cytotoxicity [26, 114, 115]. In response, tumours have developed various mechanisms to evade NK cell-mediated cytotoxicity. For instance, tumours often secrete or express factors such as TGF-β, NKG2D-L IDO, or PGE2 [116, 117], which impair NK-cell function and hinder their infiltration into the tumour site [118]. In addition, tumours may transiently upregulate MHC-I expression in response to NK cells, enabling them to evade recognition by these cells [119–121]. Next to NK cells, γδ T cells can also target tumour cells in an MHC-I-independent manner. Indeed, De Vries *et al*. have demonstrated that γδ T cells are abundantly present in mismatch repair-deficient (MMR-d) cancers with genomic inactivation of β2M. Such γδ T cells, mainly comprising Vδ1 and Vδ3 subsets, harbour an antitumoural response to MHC-I-negative, but not MHC-I-positive MMR-d tumours. Furthermore, MMR-d tumours from patients that received ICI treatment with both anti-PD1 and anti-CTLA-4 contained more γδ T cells than before the initiation of the treatment [122]. If tumours can increase MHC-I expression in response to the presence of γδ T cells remains to be proven. Tumours thus exhibit adaptability in evading both NK and T-cell-mediated cytotoxicity, facilitating immune escape.

To rescue MHC-I expression, the type of lesion will dictate the type of effective approach. 'Hard' lesions are caused by structural genetic alterations (for example mutations in β2M or other MHC-I-related genes) and are irreversible. Gene editing appears to be the only method to reverse the mutated genes. 'Soft' lesions are caused by epigenetic and/or (post)-transcriptional dysregulation, for example, destabilization of NF-kB, IRFs, and NLCR5 transcription factors, and are usually reversible defects [75, 123]. The intrinsic reversible nature of these dysregulations provides an opportunity to restore MHC-I expression.

Therapeutic strategies to rescue MHC-I 'soft' lesions

Post-transcriptional regulation of MHC-I

Loss of MHC-I expression due to (post)-transcriptional regulatory changes in genes such as MHC-I heavy chain, β2M,

or other components of the APM, can be potentially restored [123]. Different pathways can regulate the transcription of MHC-I genes as well as other genes responsible for the APM. One potential target to upregulate MHC-I expression is the interferons (IFNs) signalling pathway. Both type I and type II IFN pathways, when activated, can induce dimerization of STAT proteins, which then migrate to the nucleus and regulate the transcription of numerous genes, including MHC-I genes [124]. MHC-I could be induced, with a more than 50% increase after exposure to IFN-γ in 14/19 human tumour cell lines [125]. Loss of MHC-I can also be attained by dysregulation of the IFN-γ pathway: defects in the IFN-γ signalling pathway melanoma cell lines were caused by the absence of STAT-1 phosphorylation. IFN-α treatment could induce STAT-1 phosphorylation and consequently MHC-I expression [126]. Such data carries relevance as IFN-γ treatment is being applied in clinical practice [127]. Adenovirus vectors with IFN-γ cDNA have been tested in clinical trials to treat cutaneous lymphoma and melanoma [NCT00394693, [128]]. Various clinical trials have been using IFN-γ as an adjuvant for vaccine therapies and chemotherapies [NCT00428272, NCT0049-9772, NCT00824733, NCT00004016]. Alternatively, inhibition of negative regulators of IFN-γ signalling could reactivate the pathway and increase MHC-I expression. Several proteins, such as double homeobox 4 (DUX4), lymphocytes adapter protein (LNK), or protein tyrosine phosphatases, have been implicated in impaired IFN signalling in cancer [129–131]. Therefore, inhibiting these proteins might be beneficial when combined with immunotherapy to increase MHC-I expression and enhance T-cell-mediated cytotoxicity. However, as many of these proteins have not been correlated directly with MHC-I expression yet, further investigation is needed.

Another potential target for MHC-I upregulation is the NF-kB pathway. The NF-kB pathway consists of many inducible transcription factors (Rel, p65, RelB, p105/p50, p100/p52), which under normal conditions are inhibited by IkBs and IKKs. After cell stimulation, NF-kB is activated and enters the nucleus to bind and transcribe target genes, including MHC-I. The NF-kB signalling pathway is a key regulator of cellular immunity, inflammation, and stress, and is furthermore involved in cell differentiation, proliferation, and apoptosis [132]. The NF-kB pathway is often altered in both solid and haematopoietic malignancies [133]. Lorenzi *et al.* showed that transfecting NB cell lines with both NF-kB p65 and IRF1 transcription factors induces optimal recovery of cell-surface MHC-I expression. They observed a critical dependence of MHC-I/APM reactivation on the NF-kB/IRF1 pathway [60]. Alternatively, as for the IFN pathway, negative regulators of the NF-kB pathway could be targeted. Previous research in our lab identified two major negative regulators of MHC-I via NF-kB signalling in NB; Nedd4 Binding Protein 1 (N4BP1) and TNF-alpha-induced protein 3 interacting protein 1 (TNIP1). Targeting NA4BP1 or TNP1 could result in strong activation of NF-kB signalling, which should substantially increase MHC-I expression [134].

Finally, a third regulator of MHC-I transcription is NLCR5 (NOD-like receptor family, caspase recruitment domain containing five). Several studies with NLCR5-deficient mice showed that NLRC5 is a key factor in the transcriptional regulation of MHC-I [135, 136]. Various expressional and functional defects of NLRC5 have been found in many cancers, associated with impaired cytotoxic T-cell activation and poor patient prognosis [137, 138]. No specific compounds targeting NLRC5 have been reported yet. However, the expression of NLRC5 is highly induced following the activation of STAT1 in response to IFN-γ stimulation [55]. Targeting pathways that regulate the transcription of MHC-I or β2M genes or other components of APP pathway, is a promising strategy to restore MHC-I expression and enhance the response to immunotherapies in cancer patients.

Alternative strategies to upregulate MHC-I 'soft' downregulation

For cancers that have lost MHC-I expression due to epigenetic silencing mechanisms, it should be possible to restore MHC-I expression by reversing the repressive epigenetic status of the MHC-I-related genes. In support, recent work showed one such example, in human breast cancer types in which MHC-I genes were found to be methylated in some cases, suppressing the expression of those genes. Treatment with a DNA methyltransferase inhibitor (DNMT) in a murine breast cancer model upregulated MHC-I expression in tumour cells, which promoted the recruitment of CD8+ T cells to the microenvironment [139]. The prolonged treatment with a high dose of the DNA hypomethylating agent 5-AZA-2′-deoxycytidine showed the induction of de novo expression of MHC-I genes in MHC-I-negative melanoma cell lines [140]. Histone deacetylase (HDAC) inhibitors can also regulate MHC-I gene expression, inducing hyperacetylation of histones, thereby activating transcription. Combination therapy of HDAC inhibitors and ICIs is currently tested in melanoma patients and in patients with other cancer types [59, 141, 142].

Various oncogenic pathways have been reported to affect the expression of MHC-I, β2M, and other APM components in cancer, including the MAPK/ERK pathway. The MAPK/ERK pathway regulates NF-kB transcription factors and has been suggested to negatively influence MHC-I expression by decreasing IRF1 activity and STAT1 expression [143, 144]. Therefore, inhibiting the activity of this pathway might restore MHC-I expression. Indeed, it was shown that mutational activation of MAPK/ERK pathway inhibits MHC-I expression in NSCLC and contributes to the poor response to immunotherapy. Accordingly, the treatment of an NSCLC cell line with trametinib, a MEK inhibitor, increased MHC-I expression [145]. Therefore, inhibiting these oncogenic pathways that regulate MHC-I transcription might constitute another promising strategy to restore MHC-I expression.

Potential therapeutic strategies to rescue MHC-I 'hard' lesions

The MHC-I heavy chain and β2m light chain are essential for antigen presentation. 'Hard' lesions in MHC-I genes require gene replacement or gene editing to rescue and recover MHC-I expression. *In vitro*, this has been accomplished by the transfection of MHC-I pathway genes into cancer cell lines [146]. Similarly, gene therapy with a β2M-adenoviral vector has been proven successful in restoring MHC-I expression in human melanoma cell lines with β2M mutations [147]. A major challenge is the transduction of cancer cells in both the primary site and the metastases. If not all tumour cells in all locations are successfully transduced, MHC-I negative clones will proliferate and metastasize. Furthermore, gene therapy must affect cancer cells only and not damage healthy tissue. Obtaining this result is undeniably challenging with the current therapies.

Of note, in contrast to hard lesions in MHC-I heavy and light chain, genetic mutations in other components of the MHC-I antigen presentation, including the PLC members tapasin, ERp57, TAP, and calreticulin, still allow for MHC-I presentation albeit a different peptide repertoire [148]. For example, defective TAP transport leads to the presentation of an altered peptide repertoire, which contains more TAP-independent peptides. Some of these peptides may be selectively presented by TAP-deficient tumour cells and are often considered neoantigens that can potentially be recognized by the immune system [149].

Rescue MHC-I low phenotype in paediatric tumours through induced differentiation

Clinical research on mechanisms that induce MHC-I expression in paediatric tumours is still ongoing. Whether MHC-I genes are mutated or not in paediatric tumours is still largely unknown. However, considering their origin and low mutational load, MHC-I gene sequences are most probably unaltered. Therefore, there is a possibility that MHC-I expression can be induced de novo in paediatric tumours. The consensus is that paediatric tumours arise from precursor cells or ESCs. Already in the 1970s, Artzt and Jacob noted that MHC-I molecules, and β2M, are absent in undifferentiated ESCs [150]. Studies in the 1980s showed that MHC-I expression in ESCs can be induced by retinoid treatment [151, 152]. Retinoids, derivatives of vitamin A, are currently being used to treat several types of cancer, including NB and leukaemia, and are known to induce differentiation, apoptosis, and inhibition of proliferation of tumour cells [153, 154]. Retinoic acids (RA) and cholesterol induce differentiation of NB cells [155], and in NB, RA can induce NF-kB transcription factor activity [156]. All this suggests that RA induces differentiation through the stimulation of NF-kB, which has an important role in the induction of MHC-I transcriptional activation. In this way, MHC-I expression could be induced de novo and could enhance the outcome of immunotherapies in paediatric tumours. In paediatric tumours, where MHC-I genes are most likely not mutated or dysregulated, differentiation therapy might be a solution. Further investigations are needed to understand the mechanism in place and consequently find potential therapeutic strategies.

Conclusion

The MHC-I antigen processing and presentation pathway plays a critical role in inducing CD8+ T-cell responses and is essential for an effective cytotoxic T-cell response. Unfortunately, tumours often exhibit downregulation of MHC-I, which is associated with poorer treatment responses and the development of therapy resistance in various cancer types. The loss of MHC-I expression can stem from defects in the antigen presentation pathway, epigenetic silencing of gene regulatory elements, loss of transcription factors, or defects in regulatory signalling pathways. Fortunately, therapeutic restoration of MHC-I expression holds promise for improving the outcomes of immunotherapies, depending on the specific mechanism of downregulation. We here highlighted several beneficial approaches to rescue MHC-I downregulation and enhance the response to immunotherapy in adult tumours. One strategy involves targeting MHC-I transcription regulators, such as IRF, NF-kB, or NLRC5, which show potential as valuable and promising therapeutic targets. Another approach involves reversing MHC-I expression by targeting repressive epigenetic markers or oncogenic pathways that suppress MHC-I transcription. We argue the importance of applying a different therapeutic approach to paediatric tumours. In this context, inducing de novo MHC-I expression becomes crucial. Potential therapies for inducing MHC-I expression may involve activating pathways that directly or indirectly regulate MHC-I gene expression. In addition, inducing differentiation in paediatric tumours appears to be a promising strategy for improving immunotherapy outcomes and may also correlate with the upregulation of MHC-I expression. Overall, further investigation is urgently required to gain a deeper understanding of the underlying mechanisms involved in the regulation of MHC-I expression for individual patients and tumour types. Ideally, in analogy to the detection and quantification of acquired mutations at the individual cancer genome level using high-throughput methods, the screening of individual tumour material for MHC-I gene mutations or post-transcriptional changes in MHC-I genes should now be considered. Such an approach might help tailor the specificity of the treatment for a single patient and thereby improve therapeutic outcomes. As such, future research should explore novel strategies aimed at inducing MHC-I expression and identifying related pathway components to enhance the anti-tumour response, for application in immunotherapies.

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Conflict of interest

Marianne Boes is a Deputy Editor of Immunotherapy Advances and as such has been blinded from reviewing or making decisions on the manuscript. Other authors declare no other competing interests.

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