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The Mechanisms Behind Immune Cell Evasion in MYC-amplified Cancers

Author:

Lisa Medema (s3727939)

Lecturer:

prof. dr. M.A.T.M. (Marcel) van Vugt

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Abstract

Genomic instability, which involves the continuous alteration of the genome, is a hallmark of cancer and leads to genomic material in the cytosol. This genomic material is recognized by cytoplasmic DNA/RNA sensors which leads to inflammatory signaling. Due to inflammatory signaling, such as interferon (IFN) signaling, immune cells are attracted to the tumor site to clear the tumor cells (Zimmerli et al., 2021; Chen et al., 2022). In certain cancers, immune clearance is overcome through various mechanisms. The expression of immune checkpoint components, including PD-1/PD-L1 and CTLA4, by cancer cells are examples of such mechanisms (Iwai et al., 2002; Buchbinder et al., 2016). For that reason, immune checkpoint inhibitors (ICI) have been developed which are used as a treatment in different cancers. Recently, the oncogenic transcription factor MYC has been getting attention due to its properties to evade the immune system in cancers. In this essay, I will focus on the MYC oncogene and its role in immune evasion in cancers.

In non-cancer cells, MYC is a master gene expression regulator that promotes proliferation and multiple other cellular processes (Kim et al., 2008; Zimmerli et al., 2021). MYC dimerizes with MAX to promote gene expression, while this complex can also interact with MIZ1 to repress expression (Blackwood & Eisenman., 1991; Staller et al., 2001). In cancer, MYC is often affected by amplifications of other genes which leads to induction of MYC which consequently leads to immune evasion (Sodir et al., 2020). Additionally, treatment with immune checkpoint inhibitors do not work on MYC-amplified cancers. The mechanism behind the immune evasion of MYC was so far unknown.

From the previous findings, it appears that MYC achieves immune cell evasion by repressing inflammatory genes and therefore interfering with the inflammatory signaling. For that reason, restoration of the inflammatory signaling is important for treatments of MYC-amplified cancers (Zimmerli et al., 2021). Targets to achieve immune cell activation could be MIZ1 repression, the RAS pathway, degradation through FBXW7, and the MXD/MNT proteins (Muthalagu et al., 2020; Molina et al., 2006; Carroll et al., 2018; Sears et al., 2000; Allen-Petersen & Sears., 2017). Also, direct inactivation of MYC could be investigated. Compounds against MYC have already been discovered, although most are not functional in vivo. Omomyc is the best-studied peptide-based MYC inhibitor so far. This compound interacts with the MYC network thereby preventing DNA binding and heterodimerization with MAX. In mouse models of lung adenocarcinoma omomyc appears to reprogram the immune system to clear the tumor cells (Beaulieu et al., 2019). Nonetheless, clinical trials with omomyc are still to be waited upon (Madden et al., 2021).

Thus, immune evasion is the cause of aberrant MYC which represses inflammatory signaling. Targets to restore the inflammatory signaling are being researched and include MIZ1 repression, the MXD/MNT proteins as well as targeting MYC directly. From the data it is clear that MYC expression should be reduced in MYC-amplified tumors. In the future, more trials, including clinical trials, with omomyc should be done to investigate its potential as treatment for MYC-amplified cancers.

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Introduction

To date, cancer remains one of the leading causes of death across the globe. The immune system interacts with tumors over the entirety of cancer progression. The crosstalk with the immune system can lead to tumor regression or progression and the balance between the two determines the outcome (Vinay et al., 2015). If the immune system wins, tumor clearance will be achieved leading to cancer regression. As a result, many tumors have developed the ability to evade the immune system with a variety of mechanisms involved.

One of these mechanisms is the immune checkpoint comprising the programmed cell death ligand 1 (PD-L1) and the programmed cell death protein 1 receptor (PD-1). PD-1 and PD-L1 downregulate T-cell-mediated immune responses (Freeman et al., 2000). Immune checkpoints are a normal part of the immune system and prevent immune responses from being too strong. PD-1 is expressed on activated T cells, B cells, macrophages, regulatory T cells, and natural killer (NK) cells. The ligand can be present on a diverse range of cells (Franzin et al., 2020; Gong et al., 2018). In the case of cancer, PD-L1 is present on the cancer cells and after binding to PD-1 this results in the deactivation of the immune cell that has the PD-1 receptor. Thereby, the tumor can evade the immune system, allowing cancer progression (Iwai et al., 2002). Even though the PD-1/PD-L1 signaling is a way for cancers to evade the immune system and thus progress, de-activation of this signaling is a way to restore the immune system.

Apart from PD-L1/PD-1, the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is also an immune checkpoint (Buchbinder et al., 2016). CTLA-4 is a CD28 homolog which binds in a competitive manner to B7 (Chambers et al., 2001). Moreover, dimeric CTLA-4 has a higher affinity to B7 than the CD28 (Greene et al., 1996). The binding of CTLA-4 to B7 does not produce the costimulatory signal that is provided when CD28 bind to B7 (Chambers et al., 2001). The costimulatory signal from CD28/B7 binding allows T-cell activation leading to proliferation and differentiation. The relative amount of CD28/B7 binding in comparison to CTLA-4/B7 binding determines whether the T lymphocyte will achieve activation. CD28 molecules are located on T cells while B7 molecules are located on the antigen precenting cells (APCs) such as macrophages (Buchbinder et al., 2016). CTLA-4 is expressed on T regulatory cells but can also be upregulated in other T cell subsets. Furthermore, the CTLA-4 can be expressed on cancer cells (Seidel et al., 2018). With CTLA-4, cancer cells can deactivate T cells leading to immune evasion. Therefore, inhibition of CTLA-4 can restore immune cell invasion of the tumor site.

Based on these findings, immune checkpoint inhibitors (ICI) were developed. These inhibitors are monoclonal antibodies that interrupt the delivery of inhibitory signals to the immune cells such as T cells. The inhibitors can bind the PD-1 receptor or ligand (Franzin et al., 2020) as well as the CTLA-4 (Seidel et al., 2018). The use of immune checkpoint inhibitors gives survival benefit in multiple tumor types (Franzin et al., 2020; Seidel et al., 2018). The success of this type of treatment is associated with a high mutational load in the tumor cells and the involvement of tumor-infiltrating lymphocytes (TILs) (Gong et al., 2018). Nonetheless, there are tumor cases in which immune checkpoint inhibitors have limited effects, even when there is high mutational load and high levels of TILs such as in triple-negative breast cancer (TNBC) patients (Zimmerli et al., 2021). Clearly, a better understanding is needed of the mechanisms that govern a response to ICI, to achieve a more effective use of these therapeutics.

Recently, the MYC oncogene has been coined as a possible explanation for the lack of efficacy of ICI in certain cancers. As immune checkpoint inhibitors are non-functional in MYC-amplified cancers (Wu et al., 2021; Zimmerli et al., 2021), MYC appears to not use immune checkpoints to achieve immune evasion. However, the mechanism by which immune evasion is

achieved remains unclear. Therefore, in this essay, genomic instability in cancer and the consequences of expression of the MYC oncogene will be discussed. Subsequently, the mechanism behind immune system evasion in tumor cells will be discussed as well as treatment possibilities. Ultimately, this essay aims to answer how the MYC oncogene causes immune evasion in cancers with a low response to ICI and what could be targets for treatments.

Genome instability and inflammatory signaling in cancers

Genomic instability, which is defined as the inability to maintain the structural and numerical integrity of the genome, is a hallmark of cancer (Hanahan & Weinberg., 2011). This instability can comprise both structural and numeral alterations of the genome. Structural alterations include mutations and chromosomal rearrangements, while numerical alterations include the gain or loss of entire chromosomes. There are multiple mechanisms in cancer that can cause these instabilities, including oncogene-induced replication stress and defective mitotic chromosome segregation. Importantly, genomic instability clears the way for tumors to obtain oncogenic features that allow proliferation and metastasis (Chen et al., 2022)

Cells have processes that regulate DNA replication and repair and maintain genomic integrity. Important is the DNA damage response which is a group of conserved pathways that respond to damaged DNA (Chen et al., 2022). BRCA1 and BRCA2 are both involved in homologous recombination used to restore double-strand breaks (Prakash et al., 2015; Davies et al., 2001). Mutations in these two proteins highly increase the risk to develop breast and ovarian cancer (Bonadona et al., 2005). Due to these mutations, homologous recombination is defective which leads to alterations in the genome that are unrepaired or wrongly repaired by error-prone repair pathways. Consequently, these alterations lead to malignant traits that provide metastatic ability, increase immune evasion, and resistance to anti-cancer agents and immunotherapy (Chen et al.,2022). Mismatch repair (MMR) is important during and after DNA replication and mutations in one of the MMR genes, such as MutL Homolog of E. coli 1 (MLH1), lead to the accumulation of point mutations and deletions/insertions. Mutations in the MMR genes are often in repetitive sequences called microsatellites (Chen et al., 2022; Pećina-Šlaus et al., 2020). The sequence lengths withing microsatellites are altered with MMR deficiency, giving rise to microsatellite instability (MSI). MSI is a genomic instability that is a characteristic for tumor cells (Pećina-Šlaus et al., 2020). MMR defective tumors have genomic instability which is correlated with rapid tumor growth and the acquisition of drug resistance. On the other hand, these mutations are also correlated with good responses to the immune checkpoint inhibitors (Germano et al., 2018). Replication stress is the process of slowing or stalling of replicating forks (Gaillard et al., 2015). Replication stress drives genome instability and is mainly caused by oncogene expression (Tsantoulis et al., 2007). Therefore, oncogene expression, such as MYC amplification, can induce DNA damage and is linked to different genome deviations.

DNA is supposed to be localized within the nucleus and mitochondria. In the case of cancer-related genomic instability, DNA gets into the cytoplasm due to micronuclei formation. These micronuclei later rupture which releases the DNA. Nonetheless, DNA can also be released into the cytosol on its own (Chen et al., 2022). Cytoplasmic DNA can be recognized by multiple sensors including cyclic GMP-AMP synthase (cGAS), retinoic acid-inducible gene 1 (RIG-1), and Toll-like receptors (TLRs) (Chabanon et al., 2021). cGAS is part of the cGAS/STING pathway. cGAS recognizes cytoplasmic DNA which activates Stimulator of Interferon Genes (STING). STING in turn causes activation of multiple proteins, including IkappaB kinase (IKK) and TANK-binding kinase 1 (TBK1), involved in type 1 IFN signaling (Chen et al., 2022). Type 1 interferons are important coordinators of tumor-immune interactions. These interferons are cytokines that can be produced by and act on

both tumor and immune cells. Type 1 IFN can mediate an innate immune response (Musella et al., 2017). It has been shown that the inactivation of BRCA-1 and/or BRCA-2 results in STING pathway activation due to DNA accumulation in the cytosol (Parkes et al., 2016). Thus, the cGAS/STING pathway connects genomic instability to innate immune responses. Additionally, RIG-1 is activated upon detection of cytoplasmic RNA. Upon activation, it binds adapter molecule mitochondrial antiviral-signaling protein (MAVS) which activates IKK and/or TBKI and consequently type 1 IFN signaling just as in the cGAS/STING pathway (Liu & Zhou., 2019; Liu et al., 2015). Furthermore, crosstalk between the cGAS/STING pathway and the RIG-1/MAVS pathway has been described. Immune pathways involving proinflammatory cytokines, dendritic cells, NK cells and CD8+ T cells were shown to play important roles in the type 1 IFN response that is induced by genomic instability. In conclusion, genomic instability leads to inflammatory signaling that can activate the innate immune system. This inflammatory signaling has consequences for cancer cells and their environment (Chen et al., 2022).

As a result, inflammatory responses can give rise to anti-tumor activity. For example, homologous recombination deficient cancers that recognize cytoplasmic DNA/RNA showed a longer overall survival (Yan-Fei et al., 2020). The previous indicates that inflammatory responses can have an anti-tumor activity that promotes tumor clearance. Of note, long-term inflammation may lead to enhanced proliferation, immune evasion, and metastasis and thus has pro-tumor activity (Bakhoum et al., 2018). As for the anti-tumor activity, the type 1 interferon responses can act paracrine on neighboring tumor cells where it induces interferon-associated chemokines such as XCXL10. Moreover, these responses can also act on immune cells, including TILs, which activate anti-tumor immunity. Furthermore, type 1 interferon stimulates the activation of antigen-presenting cells and antigen cross-presentation to CD8+ cells (Chen et al., 2022). CD8+ cells of the adaptive immune response are the most powerful cells in the anticancer immune response. Therefore, activation of these cells is an especially important anti-tumor mechanism and used in cancer treatment (Raskov et al., 2020). With these mechanisms, the inflammatory signaling can help enhance the immune clearance of tumors.

Thus, genomic instability provides a disadvantage to tumor cells and these alterations can make tumor cells susceptible to clearance by the immune system. Therefore, tumors need to be able to overcome clearance to allow tumor progression. Different mechanisms that allow immune evasion are known and knowledge about this topic has grown in recent years (Chen et al., 2022). Immune checkpoints are commonly upregulated in cancers to reduce T-cell activation (Pardoll, 2012). To treat this immune checkpoint inhibitors (ICI) were developed which resulted in a breakthrough for cancer treatment (Chen et al., 2022; Postow et al., 2015). ICI block suppressive immune receptors and therefore revitalize deactivated immune cells (Raskov et al., 2020). However, some genome unstable tumors are still able to overcome clearance by the immune system even with ICI. This adaption can be achieved by multiple mechanisms and possibly also explains the lack of response to treatments such as immune checkpoint inhibitors. A possible mechanism is to suppress the amounts of cytoplasmic DNA which can be done by reversing the genomic instability. Another mechanism is to inhibit inflammatory signaling at the level of cytosolic DNA sensing. Yet another mechanism is the overexpression of oncogenes which can facilitate immune evasion (Chen et al., 2022). Recently, the oncogene MYC has been getting attention because of its role in immune cell evasion in genome unstable cancers that lack a response to ICI treatment (Zimmerli et al., 2021).

In conclusion, genomic instability in cancers leads to DNA/RNA outside of the nucleus. Recognition of this cytosolic genomic material leads to inflammatory signaling. Inflammatory signaling can acquire immune cells to the tumor site. This could lead to tumor cell clearance, but tumor cells have developed multiple immune cell evasion mechanisms. The oncogene MYC has been getting attention because it seems to be involved in immune evasion. In the following section, the

oncogene MYC and its function in normal cells will be discussed to provide background for the mechanism of immune evasion it possesses.

The MYC oncogene and its network

The MYC family of transcription factors is composed of a highly conserved superfamily of related basic-helix-loop-helix-zipper (bHLHZ) proteins. All the components of this network can function in gene transcription and have a highly conserved basic-helix-loop-helix-zipper which is a protein-protein interaction and DNA binding domain (Carroll et al., 2018). Besides MYC (which is also called c-MYC) there are 2 other MYC family members, called N-MYC and L-MYC (Tansey et al., 2014). Furthermore, MAX and MLX are part of this network. These two proteins can form heterodimers with the other proteins in the pathway or form homodimers with themselves. MYC can only form a heterodimer with MAX, see figure 1a, and forms these heterodimers through the HLHZ regions of both proteins (Blackwood & Eisenman., 1991). The formation of heterodimers allows the dimeric basic regions to form induced-fit helixes that recognize the symmetric DNA sequence CACGTG. This sequence is part of a general class of consensus Enhancer-box (E-box) sequences (Tansey et al., 2014; Jones., 2004). Due to this, it is likely that MAX's function is to form heterodimers and facilitate DNA recognition. MYC-MAX heterodimers bind directly to the DNA but also engage other proteins to the sequence. One of the proteins that can be engaged is the POZ domain transcription factor 'Msx-interacting-zinc finger 1' (MIZ-1) (Wieze et al., 2013). MIZ-1 binds the heterodimeric interface region and can influence the transcriptional and biological activity of MYC-MAX remarkably (Carroll et al., 2018; Vo et al., 2016). MIZ1 binds to sites containing a MIZ1 binding sequence, but MIZ1 and MYC-MAX can bind as a complex to the E-boxes (Wolf et al., 2013; Walz et al., 2014). Although MIZ-1 and MYC are both transcriptional activators, they are repressive when in complex with each other (Staller et al., 2001). Therefore, the ratio of MYC/MIZ-1 at each promotor determines whether it is induced or inhibited. Depletion of MIZ-1 leads to de-repression of multiple MYC repressed genes, indicating that MIZ-1 is required for repression of a lot of MYC target genes (Walz et al., 2014). The MYC family proteins are in a regulated balance with the MXD/MNT proteins that are also part of the network. These proteins appear to have opposite functions. MYC promotes transcription, while the MXD/MNT proteins repress it, see figure 1b (Carroll et al., 2018).

MYC itself is a transcription factor residing in the 8q24 locus that regulates global gene expression and therefore promotes the proliferation of somatic cells along with various other cellular processes (Zimmerli et al., 2021). MYC is composed of three exons from which the second and third encode most of the MYC protein. The first exon remains untranslated. However, the first exon is thought to play a role in translation control and mRNA stability. Moreover, 4 transcriptional promotors of MYC exist of which promotor P2 accounts for most of the MYC transcripts (Mohamed., 2017). MYC participates in cell cycle regulation, proliferation, apoptosis, differentiation, miRNA activation, and apoptosis (Kim et al., 2008). MYC binds to thousands of promotors and nearly all genes can have their expression increased by MYC. MYC mainly binds to promoters transcribed by RNA polymerase 2 and 3 (Walz et al., 2014). Furthermore, MYC shows a preference for binding to places in proximity to CpG islands, which are genomic regions associated with transcribed genes (Zeller et al., 2006). To summarize all previous findings, there are three features of MYC binding namely, proximity to CpG islands, promoter regions, and the presence of an E-box (Zeller et al., 2006). Recently, MYC is suggested to not be an exact on/off switch of transcription but is involved in the release of the paused RNA polymerase 2 and the amplification of gene expression (Rahl et al., 2010; Nie et al., 2012). MYC recruits complexes that promote transcription and binding of the protein leads to chromatin modification that corresponds with active transcription. This mode of action of MYC explains why the loss of MYC is not enough to stop promotors from becoming or staying active.

MYC is directly and indirectly affected by signal transduction pathways (Carroll et al., 2018). These signal transduction pathways respond to external and internal stimuli, which are mitogens in the case of MYC, and are tightly controlled (Carroll et al., 2018, Sodir et al., 2020). These mitogens include growth factors and cytokines such as 'Wingless-related integration site' (Wnt) and 'interleukin-2' (IL-2). MYC is extensively regulated on multiple levels, including its protein levels, half-life, and mRNA transport. (Carroll et al., 2018).

There are more than 1500 MYC-responsive genes. Functional classification of direct MYC target genes shows that they are widely spread across distinct categories such as metabolism, cell cycle control, and intracellular signal cascade. The previous supports the statement that MYC affects global gene regulatory networks. The largest enriched class was shown to be nucleic acid metabolism. Within this class, the transcriptional regulator genes directly up regulated by MYC are MAX, MXI1, MXD3, and MNT. All of these are part of the MYC network mentioned before which puts forward another level of regulation of this network. Other genes affected by MYC are NFkB, STAT3, Erβ, JUN, ELK-4, CEBP, and ETS1 which function in cell growth control and cell cycle regulation. MYC, together with MIZ-1, can also repress genes directly (Zeller et al., 2006). Previously CDKN2B and CDKN1A were already identified to be repressed by MYC (Mao et al., 2003). These two genes are cyclin-dependent kinase inhibitors involved in cell cycle arrest and are known tumor suppressor genes (Roberts et al., 2018). As these two genes are tumor suppressor genes it is logical that the oncogene MYC would repress them to obtain tumor progression. The study of Zeller et al., 2006 has identified more direct MYC-repressed target genes. Most of these genes have a function in intracellular signaling cascades, signal transduction, and/or B cell maturation. Significantly enriched are the transcription factors 'early B cell factor' (EBF), which is required for B cell lymphopoiesis, and ZIN3 (Zeller et al., 2006).

MYC was originally discovered as an oncogene in the genomes of certain avian retroviruses, which had obtained MYC from the cellular MYC gene through retroviral acquisition. Although MYC has a critical role in normal growth and development, it is often altered in human cancers. Multiple alterations of the MYC gene are possible, including gene amplification and translocation (Carroll et al., 2018). Nonetheless, the aberrant activation of MYC is often not the consequence of alterations in the MYC gene itself but involves its intense induction by upstream oncogenes leading to deregulation (Sodir et al., 2020). In many cancers, the tight regulation of MYC, which was mentioned previously, is lost. This loss of regulation leads to abnormal protein levels which are not downregulated by appropriate signals (Carroll et al., 2018). The deregulated MYC can still bind to MAX but then binds different low-affinity E-boxes and associates with super-enhancers to promote the expression of distinct gene subsets (Lin et al., 2012). Changes in MYC levels stimulate and repress different sets of target genes that are features of MYC-transformed tumor cells. Therefore, MYC-driven tumor cells have different sets of genes that are up and downregulated in comparison to normal cells (Walz et al., 2014). Overexpression of MYC leads to stimulation of cellular growth and proliferation which in the end gives rise to transformation and tumor formation. (Carroll et al., 2018). Apart from this MYC is shown to be involved in immune cell evasion in many tumors which improves cancer survival and progression (Zimmerli et al., 2021).

Concluding, MYC is a transcription factor that upon aberrant expression in tumors becomes oncogenic. It is part of the MYC network in which it dimerizes with MAX to form a heterodimer. This heterodimer recognizes specific E-boxes and induces gene expression. In turn, the MYC-MAX heterodimer can complex with MIZ-1 to repress gene expression. Within tumor cells it causes different sets of genes to be upregulated and downregulated than in normal cells. Cancers with MYC-amplification have the ability to evade the immune system despite their genomic instability. Treatments currently used in immune-evading cancers, such as ICI treatment have no effect on these

MYC-amplified cancers. How exactly MYC causes this immune evasion is not yet known today. Therefore, possible mechanisms for immune evasion by MYC will be discussed in the next part.

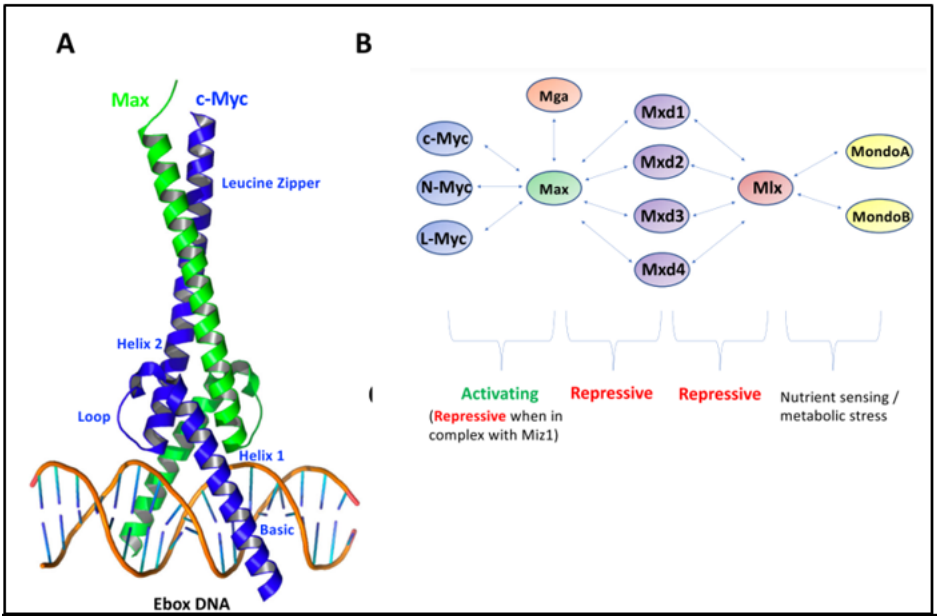

Figure 1: The structure and interaction partners of c-Myc. A Crystal structure of the c-Myc/Max dimer bound to E-box DNA (PDB ID 1NKP). B Scheme showing activating and repressing interaction partners, represented by double headed arrows, within the Myc/Max/Mxd network. Taken from Madden et al.,2021.

Immune evasion by the MYC oncogene

Aberrant and persistent MYC activation is present in most cancers (Chen et al., 2018). This oncogene is shown to influence the host tumor microenvironment and immune effectors and recent studies show a role in immune evasion (Kortlever et al., 2017). As MYC is expressed in normal proliferating cells its presence does not indicate oncogenicity. Nonetheless, overexpression of MYC is frequent in especially aggressive tumors.

Regarding MYCs immune evasion property, in different cancers with MYC amplification immune evasion was detected. For instance, pancreatic ductal adenocarcinoma (PDAC) and breast cancers that have MYC overexpression both show immune evasion (Sodir et al., 2020; Zimmerli et al., 2021). To get into more detail, a recent study by Zimmerli et al., 2021 shows that MYC reduces the number of tumor-infiltrating lymphocytes (TILs) in mice with amplified MYC compared to the mice without amplified MYC. Furthermore, immune signatures were clearly reduced in the MYC-amplified mice compared to the mice without MYC amplification. Importantly, this immune suppression appears to be local instead of systemic as in the spleen, lymph nodes, and blood no difference was observed in TILs between the mice with and without MYC amplification. Furthermore, downregulation of immune response pathways was seen in both bulk tumor samples as well as sorted tumor cells. Given these points, immune suppression is achieved in a tumor cell-intrinsic mechanism by MYC (Zimmerli et al., 2021).

To establish that the found immune evasion was indeed caused by MYC and not merely due to enhanced tumorigenesis, similar research by Zimmerli et al., 2021 was done but with MET amplified instead of MYC. The MET-amplified mice showed a similar decrease in tumor latency as the MYC-amplified mice but did not show the immune suppression MYC-amplified mice do have.

Furthermore, in another group of mice the tumor suppressor PTEN was lost in mice with and without MYC amplification to see whether it was not due to random tumor-promoting mutations. In these mice, tumor-infiltrating lymphocytes were observed in the group without MYC amplification and not in the group with MYC-amplification. Both findings indicate that MYC is selectively responsible for immune cell evasion (Zimmerli et al., 2021).

As mentioned in the introduction, immune checkpoint inhibitors are often used in cancers with high TILs (Gong et al., 2018). However, there are cancer types in which this is not effective, such as PDAC. In PDAC TILs are expulsed by MYC via the mechanism that requires macrophage-specific expression of PD-L1. PDAC is a type of pancreatic cancer in which mutated KRAS and amplified MYC leads to the progression of this cancer. It was shown that in this kind of cancer MYC activation leads to an induced PD-L1 expression on Pancreatic intraepithelial neoplasia (PanIN) cells (Sodir et al, 2020). PanIN cells are precursor lesions that eventually can lead to PDAC. In research from Sodir et al.,2020, the induction of PD-L1 by MYC could be reversed by the blockade of PD-L1. Thereby, confirming the role of PD-L1 induction in driving exclusion of T cells, such as CD3+, during the MYC-induced transition from PanIN to PDAC. As ICIs can also inhibit PD-L1, one would suggest that this is effective treatment in this case. However, the PD-L1 blockage and consequent persistence of CD3+ T cells had no inhibitory effect on PDAC transition and tumor growth (Sodir et al., 2020). Therefore, the PD-L1 mechanism is likely not the only way by which MYC causes immune cell evasion and cancer progression. Thereby, supporting the lack of impact that the ICI treatment has on the progression of cancers with MYC amplification.

Previously, it was mentioned that mutations in oncogenes, such as the BRCA genes, lead to the accumulation of cytosolic DNA which triggers the cGAS/STING pathway. This inflammation pathway leads to activation of the immune system which in principle should lead to tumor clearance. As said, tumor clearance is inhibited in MYC-amplified tumors. Therefore, there is reason to suspect that MYC affects the inflammation signaling at one point. MYC expression in breast cancer was shown to be negatively correlated with interferon and JAK-STAT signaling as well as other inflammatory pathways (Zimmerli et al., 2021). Moreover, in MYC amplified tumor models, downregulation of a variety of STING-pathway-related genes was observed. Furthermore, MYC amplification was shown to correlate with suppression of gene sets related to inflammatory signaling (Zimmerli et al., 2021) Not only in breast cancer but also in the study from Muthalagu et al., 2020 about PDAC tumors of mice with both KRAS and MYC expression (KMC) a reduction in immune cell gene expression was observed compared to tumors of mice with only RKAS expression (KC). B and NK cell presence was reduced in KMC which could be reversed by deletion of MYC. However, T-cell infiltration was not influenced by the MYC deletion (Muthalagu et al., 2020). This is in line with the previous statement that T-cell infiltration is influenced by the induced expression of PD-L1.

In the section about genome instability, it was indicated that this inflammatory signaling is particularly important in acquiring immune cells to the tumor. Suppression of the inflammatory network might therefore be the reason that the immune cells are not acquired to the tumor site. It could be that MYC inhibits cytosolic DNA, one of the mechanisms discussed in the genome instability section of this paper. However, research by Zimmerli et al., 2021 indicates that this is not the case as MYC overexpression does not rescue the increased number of micronuclei released. Therefore, it is likely that MYC works downstream of the generation of cytoplasmic DNA (Zimmerli et al., 2021). When looking at the observations from multiple studies, the regulation of gene expression might be the cause of immune cell evasion. As discussed in the MYC oncogene and its network section, MYC is a transcription factor that regulates the expression of a lot of genes. Furthermore, it was already mentioned that in cancers different sets of genes were induced and repressed by MYC (Walz et al., 2014). If we look more downstream with the study of Zimmerli et al., 2021, BRCA-1/2 depletion normally leads to increased expression of inflammatory genes such as IFNβ, IFNγ, and pSTAT1. This

amplified expression is rescued in tumors that overexpressed MYC. Furthermore, down-regulation of various STING-pathway-related genes was observed in mice with MYC-amplification. Two genes, CD74 and Ciita, which are important in the functioning of the adaptive immune response through MHC class 2 signaling are downregulated as well. When looking at enriched motif sequences, it was shown that MYC binding is specifically enriched at promoter regions of genes. Of those genes, genes involved in IFN signaling and inflammation were downregulated and corresponded to the ones found in tumors. Genes that were upregulated were also found but did not have any immunity signatures (Zimmerli et al., 2021). In the study from Muthalagu et al., 2020 about PDAC, MYC and KRAS cooperatively regulate gene expression, in which the IFN pathway via JAK-STAT was found prominently affected. At the single-gene level MYC reduces the expression of multiple IFN-induced genes, multiple IFN regulatory factors, and genes encoding STAT1 and STAT2. This reduction was (partly) present with acute activation of MYC or KRAS alone but more prominent upon the combination of the two (Muthalagu et al., 2020).

As mentioned before repression by MYC often involves MIZ1. MYC-MIZ1 attenuates the type 1 IFN cascade at multiple points that would limit both basal and IFN-stimulated gene expression. Muthalagu et al., 2020 shows that depletion of MIZ1 in PDAC models upregulated around 60% of the genes that were upregulated upon depletion of MYC from KMC cells. Both MYC and MIZ1 were found bound to promotors of STAT1, STAT2, IRF5, and IRF7 which strongly suggests that they form repressive complexes on these promotors. Moreover, the deletion of MIZ1 also restores the infiltration of NK and B cells. The recruitment of these cells was shown to involve IFN signaling which is restored upon deletion of MIZ1 (Muthalagu et al., 2020). With these findings, it is clear that MYC needs MIZ1 to repress the genes involved in inflammation.

Thus, aberrant MYC affects inflammatory signaling by regulating the expression of inflammatory genes. Most inflammatory genes are downregulated in cancer by the MYC-MIZ1 complex while upregulated genes do not show immunity characteristics. Due to the downregulation of inflammation, immune cells are depleted from the tumor site and cancers can evade the immune system and immune checkpoint inhibitors lack efficacy. Therefore, it is important to investigate new treatments with the knowledge provided. In the next section, possible treatment targets are discussed.

Therapeutic approach for MYC-amplified cancers

As stated in the introduction, current immune checkpoint inhibitors (ICI) are not effective in all cancers. A reason for this seems to be the immune evasion for which the oncogene MYC may be responsible in certain conditions. A different or additional treatment should therefore be considered in these cases. MYC is an intrinsically disordered protein and lacks druggable structural features. This makes the protein extremely difficult to target directly (Molina et al., 2006 & Madden et al., 2021). Furthermore, MYC is an important master regulator that is essential for normal cell survival and proliferation. Therefore, targeting MYC could give serious side effects in patients (Madden et al.,2021). Importantly, it has been shown that MYC targets effectors downstream in the inflammatory pathway by altering gene expression of the inflammatory genes. To achieve effective treatment the immune system must therefore be reactivated at a more downstream level. The previous could be done, although proven to be challenging, by administering interferons or targeting of MYC activity (Zimmerli et al., 2021). As said, MYC is known to inhibit IFN signaling, part of inflammation, and the insights into how this inhibits occurs should be included when designing novel therapeutic strategies that activate IFN signaling. This is of importance as reactivating IFN signaling should improve the response rates of MYC-amplified tumors that do not respond to ICI (Zimmerli et

al., 2021). Next, different possible targets of new treatments are discussed. Some are specific for immune evasion while others target MYC more generally and therefore might decrease immune evasion as well.

It has been shown by Muthalagu et al., 2020 that the targeted suppression of the MYC-MIZ1 transcriptional repressor complex by deleting MIZ1 restores IFN-related gene expression and consequently restores B and NK cell-mediated immune surveillance. Therefore, targeting MIZ1 might inhibit MYC from downregulating the inflammatory genes and restore immune invasion of the tumor. Moreover, RAS pathway inhibition was proven functional in vitro to restore IFN-related gene expression (Muthalagu et al., 2020). The RAS pathway is one of the most well described signal transduction pathways. Its role is to transduce extracellular signals to the cell nucleus where specific genes are activated. These genes can influence growth, division, and differentiation. Dysregulation of this pathway is quite common in cancer (Molina et al., 2006). RAS has been implemented in the regulation of MYC and both work together as cooperating oncogenes. RAS activation leads to a phosphorylated MYC that has a higher affinity to DNA leading to improved binding and therefore more MYC target regulation. Apart from this, the RAS network can also regulate MYC by initiating its degradation through another phosphorylation (Sears et al., 2000; Allen-Petersen & Sears., 2017). However, in cancers the RAS pathway is often mutated so that MYC degradation is inhibited while its activation is upregulated (Allen-Petersen & Sears., 2017). Therefore, inhibition of the mutated RAS is a potential target to restore inflammation signaling in MYC-amplified cancers. Nonetheless, although proven functional in vitro to use this method in vivo is complicated. Furthermore, immune cells also use this pathway to achieve their rapid expansion and in MYC-amplified cancers immune cell upregulation is very much needed. (Muthalagu et al., 2020)

As all proteins in the MYC network have relative short half-lives degradation is an important regulation mechanism. The degradation of the MYC protein is regulated by ubiquitin ligases such as FBXW7. FBXW7 requires a specific set of phosphorylation events which are stimulated by growth factor responsive signal transduction pathways. Examples of these pathways include RAS-MAPK and PI3K-AKT-GSK3β (Molina et al., 2006; Zhang et al., 2019). Inducing these pathways with growth factors may therefore upregulate MYC degradation, lessening MYCs effect. Although, as previously discusses the right part of RAS-MAPK should be induced. Furthermore, if RAS is mutated it will not degrade MYC anymore and instead upregulate MYC activity (Allen-Petersen & Sears., 2017). Induction of mutated RAS will only amplify MYC activity further which is why in this case inhibition of RAS would be wise. Moreover, the antagonist of MYC, MXD also appears to have its degradation regulated by the same pathways (Carroll et al., 2018). As MXD works opposite of MYC, its function might be beneficial in cancer treatment and should therefore not be degraded. It is therefore key to investigate which factors stimulate the pathways to degrade MYC and which factors stimulate the pathways to degrade MXD, if there is even a difference to begin with. If the pathways can be stimulated in such a way that they only degrade MYC this would be beneficial for developing new cancer treatments.

So, MYC and its family genes have an opposite function as the MXD/MNT proteins that are also part of the MYC network. This antagonism is because MXD/MNT proteins have transcriptional repression domains while MYC promotes transcription, see figure 2. The MXD/MNT proteins have been shown to retard cellular growth and proliferation and are even able to block MYC induced mitogenic effectors and transformation (Carroll et al., 2018). Therefore, using these proteins to block the effects of MYC in cancers cells might be beneficial. Inducing these proteins could induce quiescence, stopping the tumor from growing or at least slow it down. Allowing current treatment to remove the tumor. The goal would be to achieve the opposite of what is shown in figure 2b by upregulating the MYC antagonist. Furthermore, MXD/MNT and MYC compete for the available MAX (Walker et al., 2005). Overexpressing MNT as part of cancer treatment could therefore decrease

MYC-MAX heterodimers and thus diminish its effects. Zhu et al., 2008 investigated this with Mad1, encoded by the mxd1 gene, which is also a member of the MYC network. Mad1 can suppress MYC mediated transcriptional activity because it competes with MYC for heterodimerization with the partner MAX. Mad1 levels are generally low in tumors which indicates that its activity is not beneficial for tumors. Increasing mad1 levels as cancer treatment might result in repressing the MYC-mediated proliferation and transformation (Zhu et al., 2008). Whether it can block MYCs suppression of the immune system is unknown but worth further investigation as it inhibits some of MYCs pro-cancer mechanisms. Some of the other MYC-antagonists of the MXD/MNT group might otherwise block immune evasion by MYC. Nonetheless, competition for MAX of these proteins decreases MYC activity.

Currently, some therapeutics for targeting MYC are already present. Returning to the point that MYC is extremely difficult to target directly, options of direct inhibition are still being researched. So far, no small molecule inhibitors have been progressed far enough due to problems with target selectivity, rapid metabolism, and low potency. However, there are small molecules that are identified to inhibit the MYC-MAX dimerization or DNA binding. Most of these compounds were later found to not be as efficient in vivo (Madden et al., 2021). Small molecule Mycro3 has proven more promising as it prolonged survival and reduced tumor size in the KRAS-driven PDAC mouse model (Stellas et al., 2014). Furthermore, small-molecule inhibitor MYCMI-6 was indicated to selectively bind to the MYC family bHLHZ domain important for heterodimerization. This compound suppresses tumor growth in several cancer cell lines without toxicity to normal human cells as well as promotes apoptosis and reduction of tumor proliferation in a neuroblastoma xenograft model in vivo (Castell et al., 2018). Synthetic proteins, peptides, and mimetics might offer the solution to the difficult targeting of MYC. Peptides can make multiple and diverse interactions with targets that small compounds cannot (Craik et al., 2012; Mason., 2010). The downsides to peptides, such as their poor pharmacokinetic properties and low permeability, are less of a problem nowadays due to the new recombinant and synthetic chemical methods. With these methods, the problems arising for peptides can be improved. One of the proteins designed this way is omomyc which currently is the most well studied peptide-based MYC inhibitor. Omomyc has been shown to induce tumor regression in multiple cancers through increasing apoptosis and decreasing cell proliferation (Madden et al., 2021). Due to the bHLHZ chimera omomyc can bind selectively to multiple proteins of the MYC network. Interestingly, omomyc does interact with MAX, MYC, and MIZ1 but not with the members of the MXD family (Savino et al., 2011). This is beneficial as the MXD family is known as MYC antagonist and should therefore not be inhibited in treatment. Multiple mechanisms of action have been described for omomyc. First, compelling evidence suggests omomyc blocks E-box binding sites, therefore, interfering with the binding of MYC to promotor regions. Second, due to its heterodimerization with MYC and MAX, the ratio of MYC/MAX heterodimers might go below oncogenic levels. This is similar to the previously mentioned option to use the MXD family proteins to lower the MYC-MAX heterodimer level. Lastly, as omomyc prevents MYC/MAX heterodimers it could cause more free MYC. This free MYC is more likely to be ubiquitinated and degraded leading to less MYC. Beaulieu et al., 2019 indicates that in mouse models of lung adenocarcinoma, omomyc causes reduced proliferation and induced apoptosis and also leads to influx of T lymphocytes. The latter indicates an omomyc can reprogram the immune system in tumors (Beaulieu et al., 2019). Clinical trials are to be waited upon to further see its functionality in human cancer patients (Madden et al., 2021).

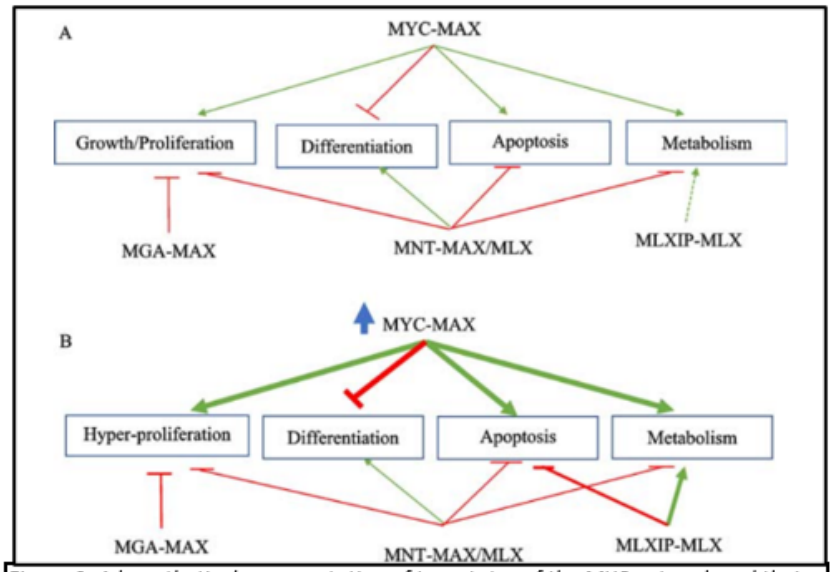

Figure 2: A hypothetical representation of two states of the MYC network and their impact on gene expression programs that influence growth, proliferation, differentiation, apoptosis and metabolism. (A) A balanced network in which gene expression is controlled through normal endogenous regulation of the MYC network. Transcriptional effects of MYC-MAX are balanced by MNT (heterodimerized with either MAX or MLX), MGA-MAX, and, under conditions of stress, by MLXIP-MLX or MLXIPL-MLX. (B) An unbalanced network due to deregulation of MYC expression. In this state MYC-MAX suppresses differentiation, reprograms metabolism, and triggers the apoptotic pathway. The suppressive effects of MGA-MAX and MNT-MAX/MLX on proliferation are overwhelmed by MYC-MAX which contributes to suppression of MYC-induced apoptosis. Increased nuclear accumulation of MLXIP-MLX (in response to deregulated MYC and/or metabolic stress) adjusts metabolic reprogramming by MYC-MAX and further reduces apoptosis. The effects on gene expression are presumed to occur through genomic binding and co-occupancy by network members. Green arrows, transcriptional activation; red arrows, transcriptional repression. Arrow width proportional to estimated transcriptional effect.
Adapted from Carroll et al., 2018.

Discussion

To conclude, genomic instability, which involves the continuous alterations of the genome, is a hallmark of cancer and leads to inflammatory signaling (Hanahan & Weinberg., 2011). Due to inflammatory signaling, such as IFN signaling, immune cells are attracted to the tumor site to clear the tumor cells (Zimmerli et al., 2021; Chen et al., 2022). In certain cancers, immune clearance is overcome through various mechanisms. Recently, the oncogenic transcription factor MYC has been getting attention due to its properties to evade the immune system in cancers. Even immune checkpoint inhibitors do not work on MYC-amplified cancers (Wu et al., 2021; Zimmerli et al., 2021). In non-cancer cells, MYC is a master gene expression regulator that promotes proliferation and multiple other cellular processes (Kim et al.,2008; Zimmerli et al., 2021). MYC dimerizes with MAX to promote gene expression, while this complex can interact with MIZ1 to repress expression (Blackwood & Eisenman., 1991; Staller et al., 2001). In cancer, MYC is often affected by mutations in other genes which leads to aberrant MYC which consequently leads to immune evasion (Sodir et al., 2020. The mechanism behind this was so far unknown. From the previous findings, it appears that MYC achieves immune cell evasion by repressing inflammatory genes and therefore interfering with

the inflammatory signaling. For that reason, restoration of the inflammatory signaling is important for treatments of MYC-amplified cancers (Zimmerli et al., 2021). Targets to achieve immune cell activation could be MIZ1 repression, the RAS pathway, degradation through FBXW7, and the MXD/MNT proteins (Muthalagu et al., 2020; Molina et al., 2006; Carroll et al., 2018; Sears et al., 2000; Allen-Petersen & Sears., 2017). Omomyc is the best-studied peptide-based MYC inhibitor so far. It interacts with the MYC network, preventing DNA binding and heterodimerization with MAX (Madden et al., 2021). Beaulieu et al., 2019 suggests that in mouse models of lung adenocarcinoma omomyc can reprogram the immune system in tumors (Beaulieu et al., 2019). Nonetheless, clinical trials with omomyc are still to be waited upon (Madden et al., 2021).

However, not all findings follow this conclusion. As discussed, research indicates that MYC depletes immune cells from the tumor site. In the study done by Zimmerli et al., 2021, it was shown that while lower fractions of monocytes, M2 macrophages, and CD8+ T cells were observed, also an increase in M0/M1 macrophages and regulatory T cells was observed. M1 is a pro-inflammatory macrophage while M2 is an anti-inflammatory macrophage. M1 macrophages are even involved in the fighting and killing of cancers cells. M2 would be beneficial for MYC as it downregulates inflammation (Orekhov et al., 2019). Thus, it is quite contradictory that MYC would acquire M1 and not M2 as this is the opposite of the function that amplified MYC has in cancers. Research done in mouse models and the overexpression of MYC is often not true to the physiological circumstances in MYC-amplified cancer in human. This deviation from the situation in MYC-amplified cancer could explain the observation that different macrophages than expected are at the tumor site.

For the future, it would be wise to have a better understanding of exactly which immune cells are excluded from the tumor, and which are still allowed by the amplified MYC as well as the function of the allowed immune cells in the tumor. Furthermore, it is important to start clinical trials with promising compounds such as omomyc that might treat MYC-amplified cancers. The targets discussed should also be investigated as these could provide new or additional treatment to ICI. Lastly, as only the findings of a few types of cancer were included in this paper it is wise to look at whether MYC works the same in other cancer types as well. An example to investigate is leukemia or ovarian cancer.

Considering all the information provided in this essay, MYC expression should be reduces. The suggestion is to continue research into omomyc as it appears promising. Next steps would be future assessment in mouse models but also clinical trials. Especially in patients with no other treatment options left, such trials can be a good option. Hopefully, with further understanding of MYC-amplified tumor and trials of MYC inhibitory compounds such as omomyc, a treatment for MYC-amplified cancers can be realized.

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