

Roles and Applications of cGAS/STING Signalling in Aneuploid Cancer

Abstract

Aneuploidy is a hallmark of cancer and affects roughly two thirds of all cancers. Normally, the DNA is well confined inside the nucleus. However, chromosomal mis-segregation as a result of chromosomal instability can cause chromosomes to end up inside separate micronuclei, which are often fragile structures. DNA leakage into the cytosol is detected by cGAS, the catalyser for cGAMP synthesis. cGAMP activates STING, which promotes the production of type 1 interferons in a TBK-1-dependent manner. Type 1 interferons are known to play a vital role in boosting both the innate as adaptive immune system, which could induce a more powerful antitumor immune response. However, cGAS/STING also features tumour-promoting abilities, such as inducing angiogenesis and metastasis. Therefore, it is highly under debate whether the cGAS/STING pathway acts as a tumour-suppressor or tumour-promotor. In this thesis, the positives and negatives of cGAS/STING signalling are put into consideration in attempt to clarify its role in aneuploid cancer. Due to cGAS/STING's ability to facilitate an antitumor immune response, this thesis also aims to inquire a possible application of the pathway in a therapeutic setting, specifically in the enhancement of immune-checkpoint blockade-based immunotherapy.

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1. Introduction

1.1 The global impacts of cancer

Cancer is the given name to a group of diseases involving abnormal growth of cells which can invade adjacent body parts or spread to other organs. Most cancers form solid masses whereas, for example, leukemias commonly do not (An et al., 2017; National Cancer Institute, 2015). Altogether, these cancers make up the second leading cause of death worldwide with roughly 9.6 million fatal cases in 2018, adding up to approximately 16 percent of all deaths, globally. Apart from the impacts on global health, cancer also forms a significant economic burden. In 2010, the total annual cost of cancer was estimated at around 1.16 trillion US dollars, and these numbers are still rising. It is predicted that the number of cancer deaths worldwide will increase by 45 percent between 2008 and 2030, due to a longer life expectancy and a growing exposure to risk factors (World Health Organization, 2019).

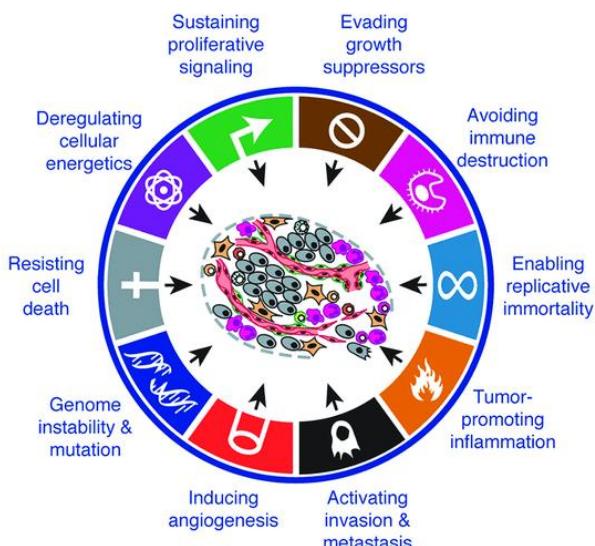


Figure 1. Enabling cancer is made possible by alterations of the hallmarks shown above. In two out of three cancers, genome instability leads to aneuploidy. Source: www.researchgate.net.

1.2 Aneuploidy in cancer

Metastasis, evasion of the immune system, resistance to cell death and an aberrant number of chromosomes are just some of the hallmarks of human cancers (Fig. 1) (Van Roosbroeck & Calin,

2017; Stopsack et al., 2019). The latter is known as aneuploidy and affects two thirds of all cancers and is the most common feature of solid tumours. (Fojer et al., 2014; Kops et al., 2005). However, in normal conditions, aneuploidy is very rare and only affects a small percentage of liver and brain cells. Aneuploidy in these cells is physiological, presumably, because of a selective favour in response to injury due to its contribution to cellular diversity. Aneuploidy in other tissues is associated with ageing disorders (Simonetti et al., 2018). Aneuploidy can be categorised in three main types: whole chromosomal aneuploidies, segmental aneuploidies (aneuploidy with a maximal length of a chromosome arm) and somatic copy number variations (gains or losses of particular regions) (Dürrbaum & Storchová, 2015). It is credible that the origin of aneuploidies during mitosis lies in the mis-segregation of chromosomes due to an impaired spindle-assembly checkpoint (SAC) function (Kops et al., 2005; Simonetti et al., 2018). SACs function is to ensure that all chromosomes are correctly attached to the kinetochores during mitosis. SAC can arrest the cell cycle in metaphase until all chromosomes are attached and aligned for mitotic progression (Rieder et al., 1995; Simonetti et al., 2018). Cells with an impaired SAC function may enter the anaphase with unattached or misaligned chromosomes, causing daughter cells to contain both copies of one chromosome or no copies at all. In many cases, these daughter cells are not viable at all. Even when they do survive, their aneuploid phenotype will have a detrimental effect on their ability of further proliferation (Williams et al., 2008). Paradoxically, aneuploidy is still a very common hallmark in most cancers. Thus, life with aneuploidy does exist. However, this phenomenon comes with both advantages and disadvantages for tumour growth.

On the one hand, somatic copy number variations can cause an elevated production of reactive oxygen species by evoking a proteotoxic stress response due to an unbalanced cellular protein composition (Dephoure et al., 2014). Moreover, a high level of chromosomal instability (CIN) is observed to be lethal for cells (Boland et al., 2009). These findings suggest a credible

disadvantage of aneuploidy for every type of cell. On the other hand, a study has indicated that there is an optimal level of CIN which is beneficial for cancer progression (Komarova & Wodarz, 2004). Aneuploidy, as a result of CIN, is involved with antigen processing, metabolism of the membrane, DNA replication and transcription, expression of major histocompatibility complex proteins and expression of cell cycle-associated proteins (Simonetti et al., 2018). This involvement differs between cancer types and aneuploid cell lines, but it can lead to a more potent and resilient tumour cell.

1.3 Cancer treatment

Presently, it is possible to approach tumours in a variety of ways. There are non-specific cancer treatments, e.g. surgical procedures, chemotherapy and radiotherapy. These ‘classic’ non-specific cancer therapies are known to be quite cytotoxic. Due to their aspecific properties, modern-day chemotherapy regimens do not only target tumour cells, but healthy tissue is being damaged as well. In addition, these treatments have shown to cause a long list of awful and harmful side effects, such as hair loss, nausea, neuropathy and infertility (Brockelmann et al., 2018; Young & Arif, 2016). However, avoidance of side effects of radiotherapy and chemotherapy can be obtained by the use of a more specific treatment method; immunotherapy.

Tumour cells have the ability to evade cell death induced by cytotoxic T lymphocytes (CTLs), and thereby making the immune systems efforts almost futile (Wein et al., 2017). The main goal of using immunotherapy is to induce an antitumor T cell reaction to immunise patients against tumour-specific antigens (Kimiz-Geboglu et al., 2018). Thereby, re-activating the immune system which was previously silenced. Immunotherapy has proven itself in numerous cases where it gave a considerable response when other treatments failed (Farkona et al., 2016).

1.4 Detection of aneuploidy by cGAS

Cyclic GMP-AMP Synthase (cGAS) was firstly described by Sun et al. (2012) as a detector of dsDNA, which can trigger a host immune response

after a viral infection or DNA transfection. However, cGAS does not discriminate between self- and non-self-DNA. Under normal conditions, all DNA is well separated from the cytoplasm within the nucleus to prevent an autoimmune response (Mackenzie et al., 2017). However, due to SAC impairment, mis-segregation is possible during mitosis and a lagging chromosome can be excluded from the actual nucleus. This causes the lagging chromosome to be entrapped in its own, often fragile, nuclear membrane and forms a structure called the micronucleus (Nassour et al., 2019). In cancerous conditions, with a moderate level of CIN and an overall genetic chaos, the micronucleus can break down, thereby releasing the chromatin into the cytosol. There, the cytosolic DNA is detected by cGAS. When cGAS was discovered, it was localised in the cytoplasm (Sun et al., 2012). However, a recent study states that cGAS is a plasma membrane protein (Fig. 2) that binds to the sugar-phosphate backbone of DNA (Barnett et al., 2019). This binding causes cGAS to form a dimer, which catalyses the production of cyclic GMP-AMP (cGAMP), thereby inducing the expression of type 1 interferons (IFN) through STING (STimulator of INterferon Genes) (Barnett et al., 2019; Ishikawa & Barber, 2008). Subsequently, type 1 IFNs can fulfil their role in activating the innate immune system and facilitate cross-priming and infiltration of T cells (An et al., 2019).

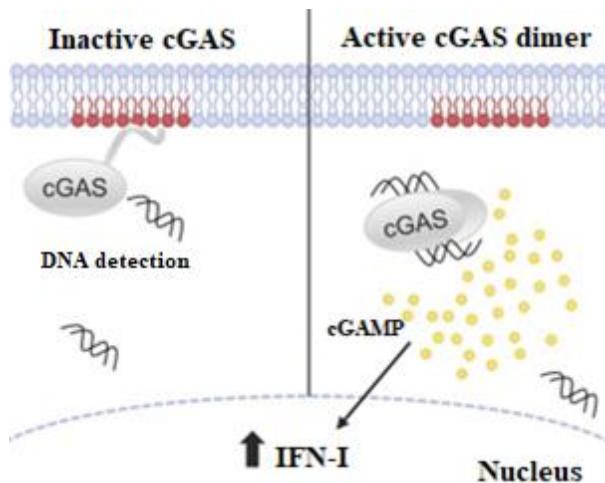


Figure 2. cGAS is a plasma membrane-bound enzyme that sensors DNA in the cytosol. Upon DNA-binding, cGAS forms a dimer and catalyses production of the secondary messenger cGAMP. Adapted from Barnett et al. (2019).

1.5 Aim of this thesis

As stated earlier, around two thirds of all cancers feature aneuploidy. Although aneuploidy can have serious negative effect on cell survival and proliferation, tumour cells are capable of turning the aneuploid tables in their favour. The cGAS/STING pathway plays an extensive role in the detection of cytosolic DNA. However, it is currently highly under debate what this role in cancer is (An et al., 2019). Therefore, the main aim of this thesis is to shed light on the precise role of cGAS/STING signalling in cancer. Furthermore, this thesis will inquire whether cGAS/STING signalling has applications for immunotherapy and if it could form an adequate co-effector in immunotherapy.

2. Immune response and cancer

2.1 The innate and adaptive response

The human immune system consists of two main immunity strategies: the innate immune system and the adaptive immune system. They differ in many ways; from cell types to duration of the response. However, these systems closely interact with each other.

The innate immune response comprises anatomic and chemical barriers, the complement system and cellular components. The cellular component of the innate immune system acts when inflammatory inducers are detected. In its broadest sense, the innate immune system is active in the presence of pathogens and tissue damage. The latter is of upmost importance for the innate response in cancer. Sensor cells, such as dendritic cells (DCs), carry receptors to detect molecules that, normally do not appear in the extracellular space or are non-self. These receptors are called pattern recognition receptors (PRRs) and detect pathogen- or damage-associated molecular patterns. Two types of PRRs can be distinguished: transmembrane PRRs, such as the Toll-like receptors and cytoplasmic PRRs, such as the NOD-like receptors. These detect extracellular and intracellular pathogens and damage, respectively. Every cell presents antigens as an indication of what is going on within it. Tumour cells are relatively fast replicating cells and

usually carry many mutated genes (Stopsack et al., 2019). Expression of these genes can lead to the production of proteins that differ from their normal version. When these variations on proteins are processed and displayed as antigens, they are recognised as non-self by the immune system. Tumour cells displaying these antigens are then targeted by natural killer (NK) cells, which are non-specific effector cells of the innate immune system. NK cells find their way to the tumour due to inflammatory cytokines secreted by other cells of the innate immune system. Activation of the innate immune system usually takes place after a couple of minutes and the response lasts for several days (Murphy & Weaver, 2016).

In contrast, the adaptive response takes days to develop, but is capable of highly specific immune responses, which is a lacking feature of the innate immune system. However, the adaptive immune system is deeply depending on the native immune system. DCs that encountered tumour cells displaying unfamiliar epitopes, travel to lymph nodes where they can activate naïve lymphocytes (B and T cells). These lymphocytes then further differentiate into fully operational effector lymphocytes. CTLs travel to the tumour environment and recognise the epitope, which is bound to major histocompatibility complex (MHC) class 1 molecules (Murphy & Weaver, 2016). After recognition, and co-stimulatory signals, the CTL releases cytotoxins, such as perforin and granzymes (Peters et al., 1991). CTLs can also use a cell-cell contact approach by inducing apoptosis via FAS-FASL interactions (Owen-Schaub et al., 1992).

In summary, the innate and adaptive response interact closely with each other. Although they use similar cell destruction techniques, they differ immensely in specificity. DCs play a vital role in presenting tumour antigens with deviant epitopes to cells of the adaptive immune system. After activation, epitope-specific CTLs are recruited and travel to the tumour environment to kill the cancer cells. NK cells use similar mechanisms, but lack the specificity of CTLs.

2.2 Role of type I IFN in the immune response

Interferons (IFNs) were the first cytokine family to be discovered over 60 years ago by Isaacs and Lindenmann (1957) and was characterised as cytokines with an antiviral activity. The IFNs are now categorised into three types and all are important factors in case of a viral infection (Hervas-Stubbs et al., 2011). Type 1 IFNs (IFN-I) comprise IFN- α (multiple subtypes), IFN- β , IFN- ϵ , IFN- κ , and IFN- ω . Signalling of IFN-I is done through the heterodimeric interferon alpha receptor (IFNAR). Activation of IFNAR leads to a rapid response, triggering multiple signalling cascades, such as the JAK-STAT and NF κ B pathways (Hervas-Stubbs et al., 2011). The antiviral implications of IFN-I are well known, but IFN-I also plays a key role in the immune response against tumours. IFN-I represses oncogene expression and increases the expression of tumour suppressor genes at the same time (Pylaeva et al., 2016). Moreover, IFN- β has proven to act as an inhibitor of angiogenesis through downregulation of VEGF (Takano et al., 2014). Furthermore, IFN-I enhances antigen recognition by increasing the expression of MHC-I. IFN-I also influences the cellular components of both the innate as adaptive immune system.

As for the innate immune system; IFN-I has shown to modulate neutrophil activation, differentiation and migration. Neutrophils are a double-edged sword as they contain both tumour-promoting as tumour-inhibiting properties. IFN-I causes a shift to the antitumor phenotype of neutrophils. On top of that, neutrophils also obtain a longer lifespan when exposed to IFN-I (Pylaeva et al., 2016). NK cells are also influenced by IFN-I due to the IFN-I-associated secretion of IL-15, which plays a vital role in the proliferation and survival of NK cells (Nguyen et al., 2002). IFN-I also affects monocytes and macrophages, causing monocytes to differentiate into DCs. Thereby, IFN-I aids the processing and presentation of antigens to T cells of the adaptive immune system (Hervas-Schubbs et al., 2011).

As stated above, IFN-I has an indirect influence on the adaptive immune system through its influence on antigen presentation by DCs. These

effects extend to CD8+ T lymphocytes, which achieve efficient cross-priming after exposure to IFN- α -treated DCs. Apart from its indirect effects, IFN-I also has a direct effect on several cells of the adaptive system. For example, B cells are directly affected by IFN-I. IFN-I presence enhances the primary antibody response, induces a long-lasting antibody production and promotes immunological memory. IFN-I further enhances antibody responses through stimulation of CD4+ T cells (Hervas-Schubbs et al., 2011).

To summarise, the effects of IFN-I range from cellular to humoral immune responses and IFN-I is able to affect the cancer cell proliferation and angiogenesis. Thus, it is clear that IFN-I plays a key role in not only antiviral responses, but also in antitumor responses.

2.3 Immune evading strategies of cancer

Tumour cells have the ability of exploiting multiple factors to avoid immune detection and destruction. One of the methods tumour cells use, is altering the tumour microenvironment to an immunosuppressive state. Tumour cells secrete immunosuppressive cytokines, such as IL-10 and transforming growth factor β , which promotes the conversion of CD4+ T lymphocytes into suppressive regulatory T cells (Vinay et al., 2015; Wein et al., 2017). Also, secretion of VEGF-A shows suppression of DCs by inhibiting their maturation process (Hanks, 2016). Another way to escape immune destruction is by upregulating or downregulating immune checkpoint-related surface proteins, such as PD-L1. Upregulated PD-L1 in tumour cells causes an inhibition of CTL activity, thereby preventing cell death. Downregulation of death receptors, such as FASR, can lead to evasion of ligand-mediated killing by CTLs. Furthermore, tumour cells can interfere with their antigen processing machinery. Downregulation of MHC-I results in a diminishment of tumour antigen presentation, which leads to impaired recognition by CTLs (Vinay et al., 2015; Weinberg, 2014).

To sum up, tumour cells can influence their microenvironment by secreting immunosuppressive cytokines. Altering the expression of components involved in antigen processing is

another common strategy to escape immune surveillance. Aneuploid tumours are dealing with cGAS/STING signalling, which can trigger a robust immune response. The functions of the cGAS/STING pathway and its role in the antitumor immune response are highlighted below.

3. cGAS/STING signalling

3.1 Functions of cGAS/STING signalling

Activation of the cGAS/STING pathway is a common manner for a cell to signal the immune system that it contains DNA in the cytosol. cGAS binds the sugar-phosphate backbone of DNA, thereby it does not depend on a specific DNA sequence. This feature makes cGAS an essential detector and regulator in many pathogenic infections (Barnett et al., 2019). Moreover, cGAS plays a key role in immune response regulation in the presence of cytosolic self-DNA. Binding of DNA to cGAS triggers a cascade that ultimately leads to the production of IFNs-I and other pro-inflammatory cytokines to give a boost to the immune system (Bose, 2017). IFN-I has proven to be of great significance in both the antiviral as antitumor immune response (see 2.2).

3.2 IFN-I production by cGAS/STING activation

When a chromosome fails to be incorporated into one of the daughter nuclei during mitosis, it forms a micronucleus (Bochtler et al., 2019). Usually, the nuclear envelope of a micronucleus is rather fragile (Nassour et al., 2019). When the nuclear envelope breaks, the DNA is released into the cytoplasm. As it drifts through the cell, it will be detected by cGAS, which is located in the plasma membrane. Activation of cGAS causes it to dimerise and the active dimer synthesises cGAMP by condensing ATP and GTP. cGAMP is the second messenger, which binds to STING, a transmembrane protein of the endoplasmic reticulum (Galluzzi et al., 2018; Ishikawa & Barber, 2008). After interaction with cGAMP, STING dimerises and recruits TANK binding kinase 1 (TBK1). TBK1 phosphorylates interferon regulatory factor 3 (IRF3), which also forms a

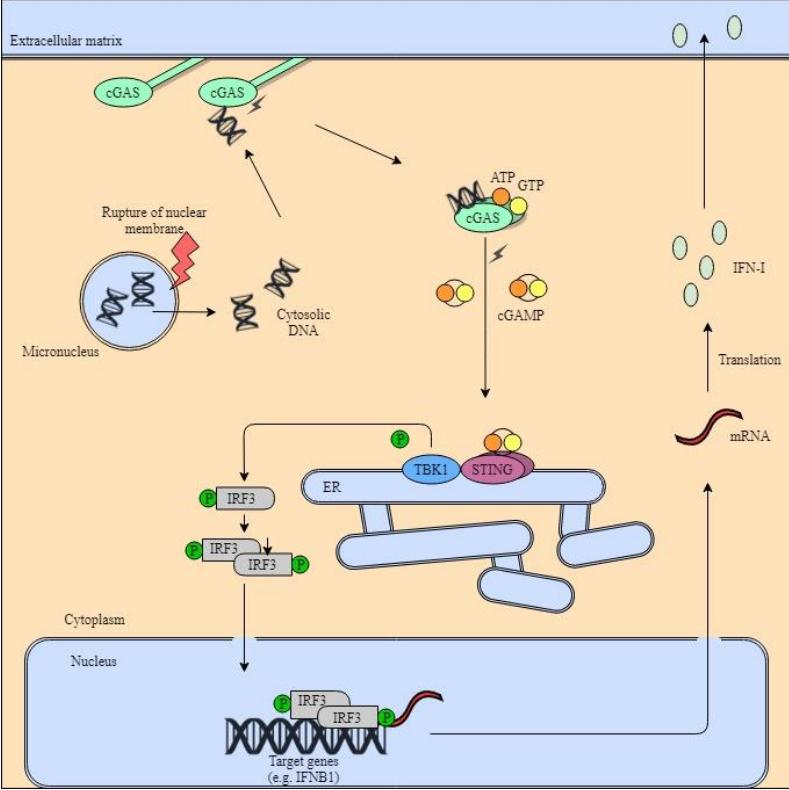


Figure 3. Schematic depiction of cGAS/STING signalling. The cGAS/STING pathway can be activated when DNA is released into the cytoplasm after breakdown of a micronucleus. cGAS catalyses the production of cGAMP, a second messenger, which activates STING.

dimer. The IRF3 dimer is imported into the nucleus where it acts as a transcription factor for IFN-I genes, such as IFN- β 1 (An et al., 2019; Galluzzi et al., 2018). Finally, translation of the RNA in the cytoplasm results in the secretion of IFNs-I (Fig. 3). It is notably to mention that there is currently a debate in the field about the exact signal transmission from STING to activation of IRF3. For instance, a study by Zhong et al. (2008) stated that an abundant secretion of IFN-I can be achieved without NF κ B activation, while others reported that NF κ B is indeed a necessary factor for IFN-I production (Abe & Barber, 2014; Ishikawa & Barber, 2008). A more recent study demonstrated that IRF3 phosphorylation by TBK1 requires the NF κ B essential modulator and IKK β (component of the IKK complex, which is a known activator of NF κ B), in addition to NF κ B activation (Fang et al., 2017). Their results implicate an important role of components of the NF κ B pathway for the phosphorylation capabilities of TBK1.

IFN- β is always expressed in a small amount, even under non-inflammatory conditions (Pylaeva

et al., 2016). However, the cGAS/STING pathway is still regulated by multiple factors and is familiar with negative feedback loops. Recently, researchers observed inhibition of cGAS-mediated IFN induction by interferon-inducible human oligoadenylyl synthetase-like (OASL) (Ghosh et al., 2019). The results of this study show that the OASL gene is a target of IRF3, promoting OASL expression. OASL then specifically binds to cGAS, thereby inhibiting its enzymatic activity. cGAS activity can also be reduced due to phosphorylation or glutamylated by AKT serine/threonine kinase 1 and tubulin tyrosine ligase like 4/6, respectively. The glutamylated itself can be antagonised by ATP/GTP binding protein-like 5/6 (Galluzzi et al., 2018). Several cyclic dinucleotides (CDNs) are also capable of inducing autophagic degradation of STING. CDNs can stimulate unc-51 like autophagy activating kinase 1, which triggers autophagy of STING (Konno et al., 2013). The cGAS/STING pathway is also regulated by a feed-forward amplification loop. IFN-I is an autocrine agent, which signals via IFNAR1/2 heterodimers. STAT1/2 phosphorylation, made possible by IFNAR1/2 activation, is associated with stimulating various genes including CGAS. Additionally, STING activity can be promoted due to ubiquitination by TRIM32, TRIM56 and AMFR/INSIG1 (Galluzzi et al., 2018).

3.3 Aberrant cGAS/STING signalling in aneuploid cancers

The innate immune system has many components which aid the body in the battle against cancer. However, the innate immune system can also ‘switch’ to the side of the tumour. For instance, neutrophils have both tumour-promoting as tumour-inhibiting capabilities (Pylaeva et al., 2016). In this thesis, the main aim remains to inquire the role of cGAS/STING signalling in aneuploid cancers and answer the question whether the cGAS/STING pathway is beneficial or disadvantageous for tumorigenesis, or both.

There are many cellular components residing in the tumour microenvironment and activation of the cGAS/STING pathway does not only occur in tumour cells. Tumour cell DNA was found within

antigen-presenting cells, such as DCs, *in vivo*. *In vitro* research then showed cGAS/STING-mediated IFN- β production due to presence of tumour-cell-derived DNA (Woo et al., 2014). On top of that, DC-dependent CD8+ T lymphocyte cross-priming is abrogated in the absence of STING as is tumour rejection in mice (Ablasser & Gulen, 2016). Thus, activation of the cGAS/STING pathway in non-tumour cells can also result in a boost for the immune system. This is one example of the antitumor features of STING. Another antitumor trait ascribed to STING is its role in the regulation of antiproliferative cytokines, such as IL18. Moreover, mice that were STING-deficient showed a higher vulnerability to the development of colitis-associated colon cancer (Ahn et al., 2015). This study indicates that STING invigorates tissue repair pathways that could prevent tumorigenesis. In contrast to these outcomes, the results of another study show a high resistance to skin carcinogenesis in mice lacking STING (Ahn et al., 2014). The authors linked a diminished level of inflammatory cytokines and absence of STING to a decreased risk and development of skin tumours driven by inflammation. These studies only address the sole effects of STING, excluding any possible cGAS involvement. Still, it is very likely that STING cooperates closely with cGAS (Ablasser & Gulen, 2016).

Tumours associated with viral infections have shown to interfere with cGAS/STING signalling by shaping the microenvironment to a more viral-favourable setting (Ablasser & Gulen, 2016). For example, E7 from the DNA tumour virus HPV binds to STING to stop its activity, thereby impairing viral DNA detection and overall cGAS/STING signalling (Lau et al., 2015). Other tumours may also use affect the cGAS/STING pathway as a way to evade an aneuploid-related immune response. The linkage of CIN, micronuclei and cGAS/STING signalling, also reveals a protumour role of this pathway. CIN has shown to promote metastasis by sustainment of a cell-autonomous response to cytosolic DNA detection by cGAS (Bakhoun et al., 2018). The same study uses aneuploid tumour models with induced CIN, which promoted cellular invasion in adjacent tissue and metastasis in a manner dependent on STING.

The cGAS/STING pathway also promotes brain metastasis of lung and breast tumour cells in a cell-autonomous and non-autonomous manner (Khoo & Chen, 2018). As noted before, many studies only report the effects of downward STING-signalling in cancer. However, a recent study by Liu et al. (2018) shows a direct effect of cGAS on DNA repair and tumorigenesis. The authors describe a DNA-damage-associated nuclear translocation of cGAS, recruitment to double-stranded DNA breaks and interactions with PARP1, thereby suppressing homologous recombination. In support of this, a knockdown of cGAS showed to reduce DNA damage and tumour growth.

Still, cGAS/STING is a widely accepted tumour suppressing signalling route and it is hypothesised that some, maybe all, components of the pathway are inhibited in the majority of cancers. A correlation between lowered cGAS expression and decreased survival of patients suffering from lung adenocarcinoma has been observed (Yang et al., 2017), supporting this hypothesis. Yang et al. (2017) also state that numerous tumour cell lines have lost cGAS and/or STING expression, which is consistent with cGAS/STING's tumour-suppressive features. According to the authors, this could explain the loss of a senescent phenotype of advanced metastatic tumour cells. However, the correlation between downregulation of cGAS and/or STING and decreased survival does not apply to every type of tumour. For example, ovarian and gastric cancers did not show a correlation that was statistically significant. These inconsistencies lead to an alternative hypothesis, in which tumour cells display a cGAS/STING-upregulated phenotype. This hypothesis is supported by research of An et al. (2019). They determined the expression levels of four key players in the cGAS/STING signalling route: MB21D1 (encoding cGAS), TMEM173 (encoding STING), TBK1 gene, and IRF3 gene. Expression of MB21D1 and TMEM173 was positively correlated with immune infiltration, whereas the expression of TBK1 and IRF3 was negatively correlated with the infiltration of innate and adaptive immune cells. However, these expression levels failed to predict the right prognosis in any cancer included in the study (n=18). It was also surprising that the expression of

all four genes was significantly upregulated in most cancers. This is likely caused by hypomethylation of the GC island within the promotors of these genes (An et al., 2019). Unfortunately, inconsistencies between promotor methylation and gene expression were found. For example, TBK1 and IRF3 expression was significantly upregulated, but their promotors were also hypermethylated in some types of cancer. Another explanation for the aberrant cGAS/STING signalling lies in possible mutations within these four essential genes. Mutations were found in this study, but most of them resided out of the functional regions, which suggests that these mutations do not account for altered functions. This leads to the possibility that a change in expression is due to regulation at a posttranscriptional level (An et al., 2019).

In summary, the cGAS/STING pathway presents itself as a potent tumour suppressor at first sight. However, tumour cells can also exploit features of this pathway to promote metastasis and a tumour-sustaining microenvironment. No research team has found a resolute correlation between expression of cGAS/STING-associated genes and alterations in functional signalling. Thus, it remains an elusive question which role the cGAS/STING pathway plays in the growth and sustaining of tumours.

4. Roles of cGAS/STING targeting in cancer treatment

4.1 Therapeutic targeting of the cGAS/STING pathway

The many effects and features of the cGAS/STING pathway are described in the sections above. These features make this pathway extremely complicated, versatile, and, more importantly, they open doors to potential therapeutic targets. One way to exploit the effects of a disrupted cGAS/STING signalling is by looking at virus-associated tumours. As mentioned in section 3.3, viruses, like HPV, can interfere with the cytosolic DNA sensing pathway and inhibit IFN-I production. This leads to a microenvironment which is more suitable for viral activity. This microenvironment can be exploited by using oncolytic viruses, such as HSV-I, which

can target and kill tumour cells (Ablasser & Gulen, 2016; Li et al., 2019; Pépin & Gantier, 2017). Another method to use the cGAS/STING pathway as a therapeutic is combining direct or indirect promoters of STING activity, leading to IFN-I production necessary for a well-organised innate and adaptive immune response. An indirect stimulator of STING is acriflavine, a DNA intercalating molecule. Acriflavine was able to promote cGAMP production in embryonic fibroblasts of mice in a setting with increased cytosolic DNA levels. Some tumours even have the ability to transfer the synthesised cGAMP to adjacent cells, which could aid in the response to chemotherapy (Pépin & Gantier, 2017). Recently, a new carboxamide compound, called BNBC, was discovered by Zhang et al. (2019). BNBC induces IFN-I and IFN-III synthesis through STING in human fibroblasts, peripheral-blood mononuclear cells, and DCs. This implies a potential role of BNBC to activate CD4+ and CD8+ T cells. STING could also be directly innervated by multiple CDNs, such as cyclic di-GMP, cyclic di-AMP, and cGAMP. A study reported that non-canonical mammalian (2'-5') CDNs are preferred compounds for entering clinical trials to obtain a higher success rate (Bose, 2017). For example, 3',3'-cGAMP was able to induce apoptosis and tumour regression in a chronic lymphocyte leukaemia model. Another promising therapeutic is 2',3'-cGAMP, which showed a firm antitumor response and stimulation of CD8+ T lymphocytes in a melanoma and colon cancer model. 2',3'-cGAMP also synergises with TLR9 agonist, causing a reduction of tumour size, tumour cell apoptosis, and DC activation. On top of that, treatment with 2',3'-cGAMP showed an upregulation of the expression of STING and IRF3, thereby enhancing pro-inflammatory cytokine production. However, the use of CDNs has a limitation. CDNs do not possess a significant cell-membrane permeability due to their electronegativity and hydrophilic nature. Thereby, their therapeutic applications are limited (Li et al., 2019; Zhang et al., 2019).

4.2 Enhancing immune-checkpoint blockade-based immunotherapy

Many tumours consist of multiple subsets of tumour cells, which are known to have different characteristics. This leads to the possible evasion of the immune response for some of the subsets, creating a challenging setting for primary therapy. Cancer immunotherapy has become a major strategy in cancer treatment and has shown to affect tumours, which formerly succeeded in escaping immune surveillance. However, there are still issues when it comes to immunotherapy in actual therapeutic practice. Therefore, immunotherapy in combination with extra adjuvants could aid to overcome these hurdles (Li et al., 2019).

The major disadvantage of CDNs in cancer therapy is described in the section above. Despite this drawback, there are still studies concerning CDN-associated cancer immunotherapy. For instance, Zhou & Jiang (2017) suggested that cyclic di-GMP could open doors for the improvement of cancer immunotherapy. Cyclic di-GMP demonstrated that it could be used as an immunotherapeutic strategy by treating patients with one high dose of cyclic di-GMP followed by several low doses. However, many other studies are sceptical about the drug delivery features of CDNs. They state that the hydrophilicity and heterogeneity of tumour lesions are two principal barricades (Li et al., 2019; Zhang et al., 2019). Synthetic 2',3'-CDNs were used in combination with granulocyte-macrophage colony-stimulating factor, resulting in STINGVAX, a cellular cancer vaccine (Bose, 2017). Mice treated with STINGVAX showed upregulation of PD-L1. Further antitumor efficacy was observed when STINGVAX was combined with PD-1 blockade. Poorly immunogenic tumours that escaped immune surveillance even after immune-checkpoint blockade-based immunotherapy, were eradicated by this combination therapy. Moreover, in a CT26 mouse model, regression of all established tumours was observed (Fu et al., 2015). On top of that, research has shown that immune-checkpoint blockage with antibodies targeting PD-L1 and CTLA-4 loses its synergistic benefit in absence of STING (Ablasser & Gulen,

2016). These results provide a positive perspective on CDN/STING-based cancer vaccines.

5. Discussion

The main of this thesis was to inquire the exact role of the cGAS/STING DNA sensing pathway in cancer. Additionally, the potential medical applications of cGAS/STING signalling regarding cancer immunotherapy were investigated. In the sections below, the positive and negative characteristics of cGAS/STING signalling in the tumour environment are weighed up against each other.

The cGAS/STING pathway induces a sophisticated immune response through IFN-I synthesis in non-cancerous cells. Therefore, many believe that cGAS/STING signalling could also be used to boost an antitumor response by the innate and adaptive immune system. Supportive evidence regards the ability of cGAS to be secreted by tumour cells, which induces IFN- β production in DCs present in the tumour microenvironment (Woo et al., 2014). STING showed to regulate the production of antiproliferative cytokines and absence of STING increases the risk of developing colitis-associated colon cancer (Ahn et al., 2015). These studies indicate that cGAS/STING plays a clear role in inducing an antiproliferative setting in cells. Therefore, it seems logical that tumour cells inhibit its signalling. Multiple (virus-associated) tumour cell lines also display this phenotype (Ablasser & Gulen. 2016; Yang et al., 2017). However, there are counterarguments, which make a good case as well. A STING-dependent promotion of adjacent tissue invasion and metastasis was observed in aneuploid tumour models with induced CIN (Bakhoum et al., 2018). Moreover, cGAS can work independently of STING to suppress DNA repair and promote tumorigenesis (Liu et al., 2018). A study which aimed to extensively investigate cGAS/STING expression could finally end the discussion. However, correlations between expression of key components of the cGAS/STING pathway and patient survival were very inconsistent. Promotor methylation typing also resulted in unpredictable situations. Therefore, it is suspected that

cGAS/STING activity in tumours may be regulated at a posttranscriptional level (An et al., 2019). The unclarity of the situation demands further investigation regarding the determination of cGAS/STING activity in different types of cancer. Knowing whether a type of cancer inhibits cGAS/STING activity or not may be vital for efficient cGAS/STING-associated treatment.

An example of cGAS/STING-associated treatment is the use of CDNs to activate this pathway in order to induce an enhanced immune response due to IFN-I production. Previous studies showed promising effects of CDNs as therapeutics (Bose, 2017; Li et al., 2019; Zhou & Jiang, 2017). Unfortunately, the hydrophilic and electronegative properties of CDNs are two major setbacks for unaccompanied CDN use for actual medical usage. Therefore, more research is required to investigate efficient drug delivery methods or the point of focus must shift towards combination therapy. The cancer vaccine STINGVAX includes synthetic 2',3'-CDNs and was able to enhance immune-checkpoint blockade-based immunotherapy in mice. The use of CDN-associated cellular cancer vaccines shows promising applications and it would be interesting to see the effects in a clinical study.

In addition to direct antitumor effects of cGAS/STING activation, the pathway may also be useful in affecting tumour growth and proliferation indirectly. After possible reactivation of the cGAS/STING pathway, IFN-I is produced by the tumour cell. IFN-I binds IFNAR1/2 in an autocrine manner, which activates IFN-I signalling pathways, such as the PI3L and NF κ B route (Galluzzi et al., 2018; Hervas-Stubbs et al., 2011). Eventually, the signal reaches the I κ B kinase enzyme complex. Besides regulating NF κ B activity, I κ B kinase also controls expression of three important pro-autophagic genes; LC3, ATG5, and Beclin-1 (Comb et al., 2011). Some cancer therapies benefit from or require activation of autophagy (Thornburn et al., 2014). It may be that the cGAS/STING could play a role in enhancing such therapies. However, the (indirect) effects of cGAS/STING signaling on autophagy have not been investigated. These possible applications of cGAS/STING could be promising but there are limitations for an actual therapeutic use, as the majority of cancer therapies

benefit from autophagy inhibition (Thornburn et al., 2014).

In conclusion, the cGAS/STING pathway plays a key role in detecting cytosolic DNA in aneuploid cells. Normally, activation of this pathway triggers and aids the innate and adaptive immune response in order to eliminate cells with a deviant number of chromosomes. However, roughly two thirds of all tumours feature aneuploidy. The cGAS/STING pathway has shown to contain tumour-suppressive as well as tumour-promoting properties, which could explain why aneuploid cancers are remarkably common. The majority of aneuploid cancers display deactivation of one or more

components which are important for cGAS/STING signalling. Therefore, the cGAS/STING pathway is generally accepted as a tumour suppressor. However, research is required to investigate the regulation of cGAS/STING components after transcription to achieve cGAS/STING-activity profiles for multiple types of cancer. Usage of CDNs included in STINGVAX has shown to be a promising candidate for the enhancement of blockade-based immunotherapy in a clinical setting. A novel application of the cGAS/STING pathway may lie in autophagy-dependent cancer therapies as cGAS/STING-induced IFN-I production could induce autophagy in tumour cells.

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