Sympathetic neurons as a new target in cancer treatment

How does the binding of norepinephrine to β-receptors in the tumour micro-environment influence cancer progression and how can this be targeted in cancer treatment?

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Abstract

Nerves from the sympathetic system are present in the tumour micro-environment. In the recent years, there has been a growing interest in their function. Sympathetic nerves are able to influence the tumour micro-environment by releasing norepinephrine. Norepinephrine binds to β-receptors. It has been demonstrated that a high density of β-receptors is present in the micro-environment. How this binding influences cancer progression and how this can be targeted in cancer treatment is discussed in this paper. Overall, sympathetic stimulation has a negative impact on cancer progression. This is caused by the activation of several downstream pathways. It was found that norepinephrine promotes perineural invasion, angiogenesis, metastasis and immune suppression. Moreover, norepinephrine is able to activate a positive feedback loop that results in the recruitment of more sympathetic nerves. Since norepinephrine has a negative impact on cancer progression, it is emerging as a potential target in cancer treatment. Several techniques are currently being studied. Sympathetic denervation, either through surgery or genetic manipulation, reduces norepinephrine release. A β-blocker can be used to inhibit the β-receptor in the tumour micro-environment. Lastly, a Trk inhibitor might be able to inhibit the recruitment of new sympathetic nerves. However, there are limitations to each of these inhibitors. More research is needed to concluded if these techniques can be used in clinical practice. Future research could focus on gaining more knowledge about the factors that influence the effect of β-blockers on cancer progression.

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Introduction

With cancer being one of the leading causes of death worldwide, cancer research has always been of great importance. In the past, researchers mainly focused on finding the genetic changes that can cause the development of cancer. In recent years a growing interest in the micro-environment of tumours has developed. Tumours are extensively connected to their environment, the so-called tumour micro-environment. This niche consists of extracellular matrix, myofibroblast, fibroblasts, neuroendocrine cells, adipose cells, immune inflammatory cells and the blood and lymphatic vascular networks (Chen, et al., 2015). A dual relationship exists between tumour cells and their micro-environment, where they both can have an effect on each other.

One component of this micro-environment are nerve fibres. In tissues other than the brain and spinal cord these are nerve fibres from the peripheral nervous system, which can be subdivided into somatic- and autonomic nerves. The autonomic nervous system exerts involuntary control over several organs and glands including the heart, blood vessels and adrenal gland. In times of stress, either psychological or physical, the sympathetic system is stimulated leading to several effects, which together are called the “fight-or-flight” response. The main neurotransmitters of the sympathetic system are norepinephrine (NE) and epinephrine (E), which have the ability to bind to β-adrenoreceptors located on the organ or gland it innervates.

Using immunohistochemical staining it was found that several cancer types including breast, lung and colon exhibit higher levels of β-adrenergic receptors compared to their surrounding healthy tissues (Rains, Amaya, & Bryan, 2017). NE released from nearby sympathetic nerves can stimulate these receptors, which is associated with worse disease prognosis (Cole, Nagaraja, Lutgendorf, Green, & Sood, 2015). Studies in prostate cancer show that injury of the spinal cord, where a functional denervation of the prostate occurs, led to decreased incidence of prostate cancer (Rutledge, Jobling, Walker, Denham, & Hondermarck, 2017). These findings support the idea that tumours are dependent on nerves and that nerves can help tumours to progress.

As of today, it is well established that sympathetic stimulation has a negative impact on cancer progression. However, there is still a lot that remains to be discovered. It is for example not clear how to translate the findings into practice. This essay describes some of the findings on the role of nerves in the tumour micro-environment and how this can be used to improve disease outcome. This leads to the following research question: “How does binding of

norepinephrine to β-receptors in the tumour micro-environment influence cancer progression and how can this process be targeted in cancer treatment?”

The β-receptor and its signalling pathway

Sympathetic signalling

The sympathetic system is activated in times of stress. This will cause the release of two main hormones, epinephrine and norepinephrine (NE). Epinephrine is mainly released from the adrenal glands, after which it circulates through the bloodstream to its target organs. NE can also be released from the adrenal glands. However, most NE acts as a neurotransmitter instead of a hormone and is released from sympathetic nerve endings. Both epinephrine and NE are able to bind to β-receptors. In total 3 different types of β-receptors can be distinguished, the β1-, β2- and β3-receptor. It was found that the β1-receptor has an identical affinity for epinephrine and NE (Hoffman, Leitz, Oberhoff-Maass, Lohse, & Klotz, 2004). The β2-receptor has a higher affinity for epinephrine and the β3-receptor for NE. It has nowadays been well established that β-receptors are present in the tumour microenvironment and can affect tumour progression. Even though, both epinephrine and NE are able to bind to β-receptors, it was found that levels of circulating epinephrine/NE were too low to influence β-receptor signalling in the tumour micro-environment (Walker, et al., 2019). This indicates that NE released from nerve terminals is mainly responsible for the effects on cancer progression. When looking at NE specifically, it has the highest affinity for the β1-receptor, then the β2-receptor and the lowest affinity for the β3-receptor.

B-receptors in cancer tissue

Previously it was mentioned that the amount of β-receptors is higher in tumour tissue compared to its surrounding healthy tissue (Rains, Amaya, & Bryan, 2017). It is however not the case that all three receptor subtypes are overexpressed in all tumour types. Analysis of tissues from 29 cancer types revealed that each cancer type has a different β-receptor expression profile. The β1-receptor was found to be most highly expressed in pancreas

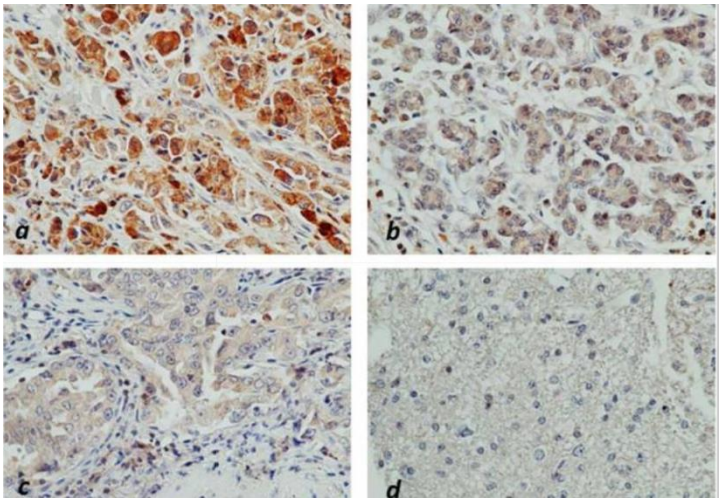

Figure 1 Staining of the β2-receptor in tissues from different cancer types. High staining of the β2-receptor in melanoma (a), pancreatic adenocarcinoma (b) and lung carcinoma (c). Little staining in astrocytoma (d). *Extracted from Rains et al. (2017)*

adenocarcinoma, melanoma, lung adenocarcinoma, clear cell carcinoma and oesophagus adenocarcinoma. The β2-receptor in pancreas adenocarcinoma, melanoma and lung adenocarcinoma (figure 1). Lastly, the β3-receptor was most highly expressed in melanoma. Overall, melanoma showed the highest expression of all three receptors. Some cancer types, like fibrosarcoma and astrocytoma showed low expression of all three β-receptors. However, most of the 29 cancer types showed high expression of at least one type of β-receptor.

The signalling pathway of β-receptors

Before the effects of sympathetic stimulation on tumour progression can be discussed, it is important to take a closer look at the β-receptor. Binding of NE to the β-receptor can lead to various effects, depending on which subtype of β-receptor is activated. All three subtypes are G-coupled protein receptors. However, the downstream signalling pathway they activate differs between the three receptors. The β1-receptor is a G*s* coupled receptor (Najafi, Sequeira, Kuster, & van der Velden, 2016). After binding of the ligand, the G*αs* subunit dissociates and activates adenylyl cyclase (AC), which results in increased cAMP. One of the effects of cAMP is activation of protein kinase A (PKA). The β2-receptor can also activate the G*αs* subunit. However, it can also be G*i* coupled. This pathway leads to decreased cAMP via activation of phosphodiesterase 4 (PDE4). Besides this, it can also activate phosphoinositide 3 kinase (PI3K), MAPK/ERK or promote the generation of nitrogen oxygen species (NOS) (Lohse, Engelhardt, & Eschenhagen, 2003). Which downstream proteins are activated depends on the tissue where the receptors are located. Even though the β2-receptor can both be G*s* and a G*i*-coupled, the majority of the time a G*αs* protein is activated (Najafi, Sequeira, Kuster, & van der Velden, 2016). Lastly, the β3-receptor can also be G*s* and G*i*-coupled, leading to the same effects as the β1- and β2-receptors (Cannavo & Koch, 2017). The signalling pathways of all three β-receptors are summarized in figure 2.

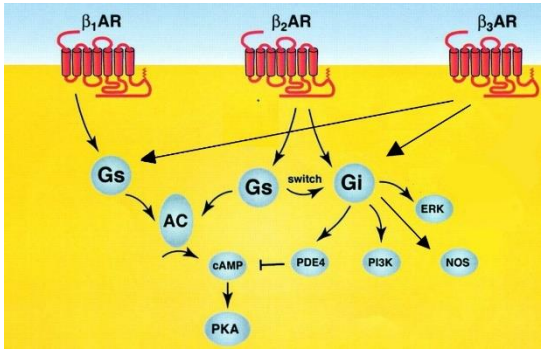

Figure 2 The signalling pathway downstream of the three β-receptors. *Modified from Lohse et al. (2003)*

The connection between sympathetic nerves and tumour cells

Sympathetic nerve denervation

The effect of sympathetic nerves on tumour progression can be demonstrated by sympathetic denervation. Denervation of a tumour can lead to reduced tumour growth. In a rat model of breast cancer, tumour size was reduced by 76% 8 weeks after surgical denervation (Kappos, et al., 2018). This denervation however, was not limited to sympathetic neurons only. Another study did specifically inhibit sympathetic nerves in breast cancer (Kamiya, et al., 2019). To achieve this they transfected mice with an adeno-associated-virus vector. This vector carried the diphtheria toxin A subunit, a protein with the capacity to eliminate neurons. Using a specific sympathetic nerve promotor, the denervation was limited to sympathetic nerves. They injected the vector into a breast cancer tumour, which resulted in decreased tumour growth. These findings support the idea that sympathetic stimulation negatively influences cancer progression. The promising results of surgical denervation in breast cancer raise the question if it might also be beneficial in humans and for other cancer types. To date, the effect of surgical sympathetic denervation in other cancer types has not been studied and remains to be explored (Zahalka & Frenette, Nerves in Cancer, 2020).

Perineural invasion

One way that tumour cells can take advantage of nerves is via perineural invasion (PNI). In this process tumour cells use nerves as a route for metastatic spread. PNI has been demonstrated in a variety of cancers including pancreatic ductal adenocarcinoma, prostate cancer, head and neck cancer, colorectal cancer and cervical cancer (figure 3) (Chen, et al., 2019). The first definition of PNI described it as “invasion in, around and through nerves” (Batsakis, 1985). Later this was changed to “A tumour in close proximity of a nerve and surrounding at least 33% of its periphery, or tumour cells invading any of the three layers of the neurolemma structure” (Chen, et al., 2019).

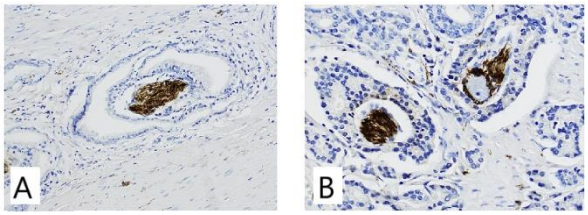

Figure 3 PNI in prostate cancer visualized by Immunohistochemical staining. It shows two classical examples of PNI, where the carcinoma cells completely surround the nerves. *Modified from Lubig et al. (2018)*

PNI is associated with poor clinical outcome. A review of 421 vulvar carcinoma cases revealed shorter overall- and progression-free survival in patients exhibiting PNI (Salcedo, et al., 2019). Similar results are found for other cancer types. One study found an association between the presence of PNI and lethal prostate cancer (Zareba, et al., 2017). Understanding the mechanisms behind PNI can be relevant in the search for new therapeutics to improve cancer. In recent years the mechanisms behind PNI are starting to be explored more. Where at first it was assumed that nerves are a passive bystander in the process of PNI, recent research reveals that PNI is a dynamic process where tumour and nerves both influence each other.

It was hypothesized by Ma et al. (2019) that sympathetic stimulation plays a role in PNI. In order to test this they used tissue samples from patients suffering from salivary adenoid cystic carcinoma (SACC). Their results showed significantly larger sympathetic nerve areas in SACC tissues with PNI compared to tissues without. Besides this, they also made other observations confirming the role of the sympathetic system. They found an overexpression of the β2-receptor and an increased NE concentration in tumour tissue with PNI. An increase in NE was found to be associated with SACC cell PNI. Blocking the β2-receptor reduced this behaviour. It was also found that increased NE results in higher expression of N-cadherin, Slug, Vimentin, MMP2 and MMP9 mRNA and a downregulation of E-cadherin. These upregulated genes are all associated with the epithelial-to-mesenchymal transition (EMT). EMT has been associated with PNI, suggesting that NE stimulates PNI by upregulating various EMT associated genes through binding with the β2-receptor.

Guo et al. (2013) found that STAT3 signalling plays a role in the effects of the sympathetic system on PNI. They conducted tests in pancreatic cancer cells and animal models, focusing on the effects of NE. They were able to show that NE increases neuronal invasion in a concentration-dependent manner. After exposure to NE, the level of phosphorylated STAT3 (the activated form of STAT3) increased, with no changes in overall STAT3 level. By using a β2-blocker it was established that increased pSTAT3 was caused by binding of NE to the β2-receptor. The administration of a β2-blocker resulted in lower levels of pSTAT3. Furthermore, they also demonstrated the effects of pSTAT3 on cell migration and invasion using a STAT3 phosphorylation inhibitor. Treatment with this inhibitor led to a decrease in PNI. It was found the effects of pSTAT3 on PNI were mediated via upregulation of MMP2 and MMP9. Both of these proteins increase the invasiveness of cancer cells.

BDNF

Besides the migration of tumour cells towards a neuron, the other way around also occurs. Tumour cells do not necessarily have to migrate to get close to a nerve. They are also able to attract nerves. The recruitment of new sympathetic nerves will lead to higher intratumoral NE levels. One of the mechanisms by which new nerves are attracted is through the release of brain derived neurotrophic factor (BDNF). BDNF belongs to the neurotrophin family of growth factors and stimulates neuronal survival and growth (Bathina & Das, 2015). Allen et al. (2018) found that BDNF was one of the most upregulated genes in NE-treated ovarian cancer cell lines. The β3-receptor played a role in this, since only inhibition of this adrenergic receptor reduced BDNF mRNA and protein levels. In agreement with this finding, a β3-agonist increased BDNF mRNA and protein expression. Downstream of the β3-receptors cAMP is increased, leading to stimulation of Epac and JNK. JNK, a transcription factor, is able to activate AP-1. AP-1 then has the ability to enhance BDNF promotor activity, and thereby increases the expression of BDNF. Higher NE thus results in higher BNDF.

To observe the effects of BDNF on tumour progression, mice were grafted with BDNF overexpressing tumour cells (Allen, et al., 2018). The mice were sacrificed after 4 weeks and the amount of nerves and tumour size were analysed. It was found that overexpression of BDNF resulted in higher nerve count and increased tumour growth compared to control. The stimulating effect of BDNF on nerve count is caused by binding to TrkB receptors on nerves. Binding of BDNF to TrkB receptors will result in higher innervation of the tumour. Silencing of the TrkB receptor resulted in less tumour innervation (figure 4). This was achieved by using a siRNA molecule, complementary to TrkB mRNA. We can thus say that NE promotes intratumoral innervation through a positive feedback loop. Enhanced NE released from sympathetic nerves, stimulates the release of BDNF from tumours. This BNDF then goes back to the nerves where it causes increased intratumoral innervation by binding to the TrkB receptor.

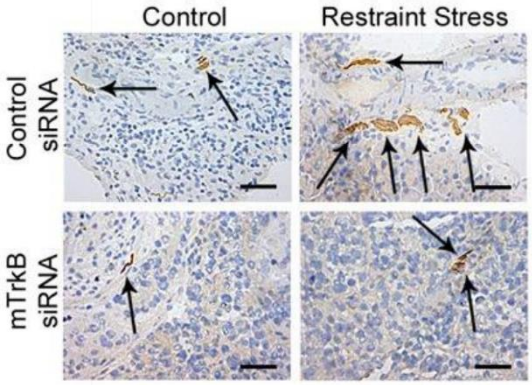

Figure 4 The effect of restraint stress and the TrkB receptor on nerve count. To demonstrate the effect of the TrkB receptor on nerve count, the receptor is silenced using a siRNA molecule. *Extracted from Allen et al. (2018)*

The effect of NE on the hallmarks of cancer

As a tumour develops it generally acquires a few specific traits. These are summarized in the hallmarks of cancer, which consist of 8 hallmarks that include the inhibition of apoptosis and sustained angiogenesis (Fouad & Aanei, 2017). Different ways to meet these hallmarks have evolved in tumour cells, which makes them, among other things, able to escape apoptosis and promote angiogenesis. The interaction of a tumour with its micro-environment plays a key role in acquiring these capabilities. Binding of NE to β-receptors on tumour cells can lead to different downstream signalling pathways that promote tumour progression. The role of NE on some of the hallmarks of cancer will now be highlighted.

Angiogenesis

Formation of new blood vessels around a tumour is required for growth and metastasis. This process is regulated by pro-angiogenic and anti-angiogenic molecules (Yang, et al., 2009). When the amount of pro-angiogenic molecules exceeds the inhibiting molecules, new blood vessels are formed. This event is also called the “angiogenic switch”. It was found that NE upregulates the pro-angiogenic factors IL-6, IL-8 and VEGF in human melanoma cell lines (Yang, et al., 2009). Treatment with NE led to increased mRNA and protein levels of IL-6, IL-8 and VEGF. This effect was mediated through binding of NE to the β1- and β2-receptor. The release of these pro-angiogenic factors activates the angiogenic switch and allows the formation of new blood vessels.

The effect of sympathetic regulation on tumour neovascularization was also demonstrated in prostate cancer. Sympathetic denervation of the prostate in a mouse model of prostate cancer, led to a reduction in size, migration and branching of blood vessels (figure 5) (Zahalka, et al., 2017). It was found that endothelial β2-receptors play a role in this. Deletion of endothelial β2-receptors, using the cre-lox system, led to a metabolic shift. It was found that Coa6, a protein involved in the electron-transport chain, was overexpressed. This overexpression increased oxidative phosphorylation and reduced glycolysis. It has been demonstrated that a metabolic shift from glycolysis towards oxidative

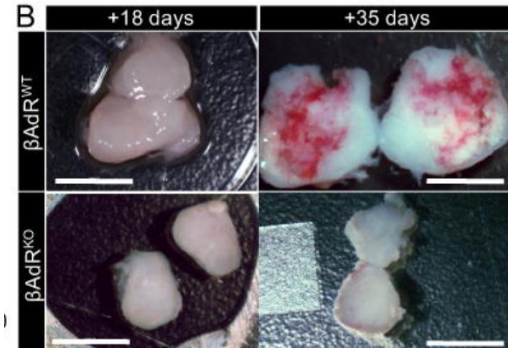

Figure 5 Tumour growth in a mouse model of prostate cancer. Tumour growth is measured after 18 and 35 days in both a wildtype and a sympathetic denervated mouse. *Extracted from Zahalka et al. (2017)*

phosphorylation reduces angiogenesis. Sympathetic stimulation thus plays a role in the formation of blood vessels by promoting endothelial glycolysis.

Invasion and metastasis

The spread of cancer cells from the primary tumour to other tissues, also called metastasis, is responsible for the majority of cancer deaths. A process associated with metastasis is the epithelial-to-mesenchymal transition (EMT). In this process epithelial cells acquire mesenchymal characteristics and become more mobile and invasive. After treating colon and lung cancer cell lines with NE, morphological changes were observed that are consistent with a mesenchymal phenotype (figure 6) (Zhang, et al., 2016). Besides this, the mesenchymal marker vimentin was increased, while E-cadherin, indicative of the epithelial state, was downregulated. It was found that these effects were induced via Snail, an important regulator of EMT. Binding of NE to β-receptors resulted in increased TGF-β1 secretion, which in turn upregulated Snail. Inhibition of the β-receptor or TGF-β1 reversed the NE induced upregulation of Snail. To inhibit the β-receptors they used the non-selective β-blocker propranolol, which is able to antagonize all three β-receptors. It can therefore not be concluded which of the three β-receptors plays a role in this process.

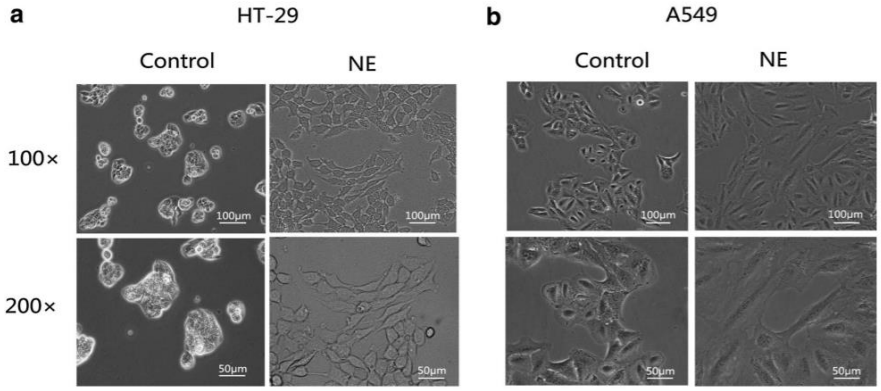

Figure 6 Treatment of HT-29 colon cancer (a) and A549 lung cancer (b) cell lines with NE. The control cells show a columnar shape, while the NE treated cells acquire a spindle-shaped phenotype. *Extracted from Zhang et al. (2016)*

Typically when cells detach from the extracellular matrix (ECM) it will result in anoikis, a form of programmed cell death. Cancer cells however, can become resistant to anoikis. This resistance allows them to survive during metastasis. Sood et al. (2010) demonstrated that ovarian cancer cells were more resistant to anoikis after NE treatment. Focal adhesion kinase (FAK) plays a key role in this resistance. Binding of NE to the β2-receptor resulted in the activation of Src. The activated Src then induced FAK phosphorylation. To verify the role of

FAK and Src in the resistance to anoikis, both were silenced. Silencing resulted in a reduction of tumour growth, demonstrating the importance of FAK and Src in this process.

Besides the protective effect of Src on tumour cells that detach from the ECM, Src is also important in other aspects of metastasis. Adrenergic stimulation with NE led to increased invasiveness of tumour cells, an effect that was abrogated by silencing Src (Armaiz-Pena, et al., 2013). It was found that levels of IL-6, IL-8, CXCL1 and BMP2 were elevated after NE treatment and went down after silencing of Src. These proteins are all known to stimulate invasion of tumour cells. Before Src is able to stimulate its downstream proteins it needs to be activated, something that can be done by binding of NE to the β2-receptor. Binding of a ligand to the β2-receptor leads to increased cAMP and activation of protein kinase A (PKA). In the case of Src, PKA is then able to induce Src phosphorylation at position S17. This phosphorylation is required for the following phosphorylation at position Y419, which activates Src (figure 7). Two of the upregulated proteins, IL-6 and IL-8 have already been discussed. Besides increasing invasiveness they stimulate angiogenesis. It could be possible that the results found by Yang et al. (2009) are mediated by the same pathway described here. If this is the case, Src not only leads to resistance to anoikis and increased invasiveness of tumour cells, it also leads to increased angiogenesis.

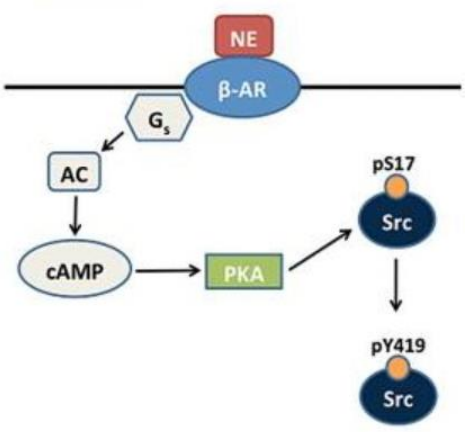

Figure 7 The downstream pathway activated by binding of NE to the β-receptor leading to phosphorylation of Src. *Modified from Armaiz-Pena et al. (2013)*

Tumour associated macrophages

While macrophages are part of our immune system, they can also promote tumour growth and metastasis. Instead of killing tumour cells, macrophages can become an essential part of the tumour micro-environment where they actually help tumour cells to progress (Mantovani, Marchesi, Malesci, Laghi, & Allavena, 2017). Macrophages thus play a dual role in cancer, where on the one hand they are able to kill tumour cells while on the other hand they can promote tumour progression. The macrophages present in the tumour micro-environment are mainly derived from the blood. Blood monocytes are recruited towards a tumour site in response to various chemo attractants. Once inside the tumour site they differentiate into tumour associated macrophages (TAM). It has been shown that TAM can influence almost all aspects of tumour progression including angiogenesis, inflammation, EMT and metastasis.

One study found that elevated levels of NE, through restraint stress, resulted in a higher amount of tumoral macrophages (figure 8) (Armaiz-Pena, et al., 2015). In ovarian cancer cells this worked via the production of monocyte chemoattractant protein-1 (MCP1). The β-receptor-cAMP-PKA signalling axis is responsible for this increased production. To test which β-receptor plays a role, several antagonists were administered. Only propranolol and ICI115,881 (β2-blocker) were able to prevent MCP1 production. It can therefore be concluded that the β2-receptor is important in this pathway. Specifically the G*as* coupled receptor, since the G*as* subunit stimulates cAMP and PKA.

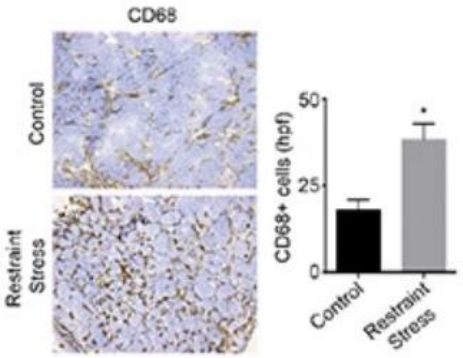

Figure 8 Immunohistochemical staining of tumour samples demonstrating the effect of restraint stress on the number of CD68 macrophages.
Extracted from Armaiz-Pena et al. (2015

Macrophages can be divided into two categories, M1 and M2 macrophages. The latter is associated with immunosuppression and increased angiogenesis by release of IL-10 (an immunosuppressive cytokine) and VEGF (promotes angiogenesis). The M1 phenotype is associated with inflammatory cytokines, and therefore less favourable in a tumour micro-environment. It has been demonstrated that a high M1/M2 ratio is associated with increased survival of ovarian cancer patients (Zhang, et al., 2014). This ratio is influenced by sympathetic signalling. Release of NE induced a shift in macrophages towards a M2 phenotype in lung cancer (Xia, et al., 2019). The M2 associated gene Arg-1 was upregulated after NE treatment. Besides this, higher levels of IL-10 and VEGF were observed, which is associated with immune suppression and increased angiogenesis.

Therapeutic implications

B-blockers

B-blockers are β-receptor antagonists and therefore prevent the binding of NE. They are commonly used to treat hypertension and heart failure, as they lower blood pressure and heart rate. B-blockers can be divided into 2 subgroups, selective and non-selective. While the selective blockers mainly antagonize the β1-receptor, non-selective blockers inhibit the β1-, β2- and β3-receptor. It was observed that cancer patients who were using β-blockers before being diagnosed with cancer showed reduced disease progression (Cole, Nagaraja, Lutgendorf, Green, & Sood, 2015). This observation led to the idea of using β-blockers as a drug in cancer treatment. Nowadays, multiple studies have been conducted in various cancer types. A few studies specifically looked at the use of a β-blocker in the preoperative period. Removing a tumour can both be psychologically and physiologically stressful. The physiological stress, also called surgical stress, is the body's reaction to surgery. In response to this surgical trauma, the hypothalamus is activated, which in turn activates the sympathetic system (Finnerty, Mabvuure, Ali, Kozar, & Herndon, 2013). In combination with the psychological stress, this leads to an increase in sympathetic activity and thereby NE.

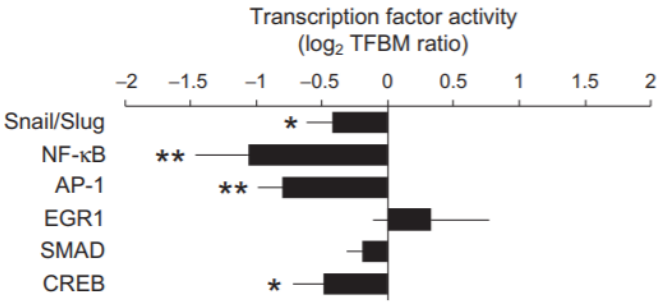

Figure 9 The effect of propranolol on the expression of various genes that are involved in the epithelial-to-mesenchymal transition. *Extracted from Finnerty et al. (2013)*

It was hypothesized by Hiller et al. (2020) that using a β-blocker during the preoperative period reduces sympathetic activity and thereby metastasis in breast cancer patients. The non-selective β-blocker propranolol was used to achieve this β-blockade. It was found that the use of propranolol for 7 days prior to surgery resulted in a downregulation of genes involved in EMT in tumour cells (figure 9). The transcription factors Snail and Smad were both downregulated. Both of these factors are involved in EMT and are normally upregulated with the transition towards a mesenchymal phenotype. Besides this, NF-κβ and AP-1 were also downregulated. These transcription factors are associated with inflammation. The transcription factor CREB, which is activated by cAMP was also found to be downregulated.

Despite the promising results found by Hiller et al. (2020), an analysis of 30,020 breast, colorectal and lung cancer patients showed no benefits of β-blocker use on survival (Musselman, et al., 2018). They particularly looked at use during the perioperative period, the period of time between ward admission and recovery. To determine if the effects were cancer type specific, all 3 cancer types were analysed separately. Still, they were unable to find any benefits of β-blocker use. When separating the use of non-selective and selective β-blockers, still no association was found.

Similar results were found in a meta-analysis of 27 studies (Yap, et al., 2018). This study not only included patients who were using β-blockers during the perioperative period but also incidental use. Overall, the use of β-blockers did not improve overall survival, disease-free survival or cancer recurrence. However, the results differed between cancer types. according to cancer type showed an increase in disease-free and overall survival in melanoma and ovarian cancer. Another meta-analysis also showed no association of β-blocker use with overall survival, overall mortality, disease-free survival, progression-free survival and recurrence-free survival (Na, et al., 2018). The only significant association was found between β-blocker use and cancer-specific survival, which is the period of time from diagnosis until cancer-related death. Analysis of cancer types separately showed longer overall survival in melanoma, ovarian and pancreatic cancer. This partially corresponds to the findings of Yap et al. (2018), who found a positive effect of β-blocker use on overall survival in melanoma and ovarian cancer, but not in pancreatic cancer. The effects of β-blocker use were greatest in melanoma. This was found in both meta-analyses. In total, this was based on three studies. When looking at these studies individually, one study reported a 13% decrease in mortality after β-blocker use (Lemeshow, et al., 2011). Another study found that 3% of β-blocker users had disease progression or died, compared to 8% in the control group (De Giorgi, et al., 2013). The third study reported that 15,8% of patients using propranolol showed disease progression compared to 41,2% in patients that did not use propranolol (De Giorgi, et al., 2017).

Chemotherapy is often used as the primary treatment of a tumour. It is therefore of interest to see what the effects of combining β-blockers and chemotherapy are. Using a drug combination study it was found that propranolol synergizes with the chemotherapeutic drug vinblastine (Pasquier, et al., 2016). Combination of these two drugs resulted in supressed growth and increased apoptosis in an angiosarcoma cell line. Based on these findings, a combination of propranolol and vinblastine was prescribed to seven angiosarcoma patients. This resulted in a median overall survival of 16 months, compared to 9 months with standard treatment. Various other studies have focused on the effects of β-blockers on chemotherapy-induced cardiotoxicity. As a result of chemotherapy, free radicals can be generated, damaging

cardiomyocytes (Huang, et al., 2019). It has been shown that the use of trastuzumab combined with anthracyclines is associated with cardiotoxicity in breast cancer. Guglin et al. (2019) conducted a randomized trial to determine if β-blockers can reduce this effect. They treated breast cancer patients with trastuzumab and anthracyclines, after which they received the non-selective β-blocker carvedilol. They found that carvedilol indeed reduced cardiotoxicity.

Targeting BNDF

Another process that can be targeted to decrease sympathetic stimulation is the recruitment of nerves. It has been demonstrated that the local release of NE from nerve endings has a bigger impact on cancer progression than circulating plasma NE (Walker, et al., 2019). The recruitment of nerves towards a tumour site is therefore extremely important. Without these nerves little NE will be present in the tumour micro-environment. We have described that BDNF plays a role in this recruitment. Besides this effect on nerves, BDNF also promotes other aspects of cancer progression. It has been demonstrated that BDNF increases proliferation, EMT, resistance to anoikis and sensitivity to chemotherapy, among other things (Meng, et al., 2019). The beneficial results of targeting this pathway will therefore not only be caused by decreased innervation of a tumour.

The BDNF signalling pathway can be targeted by inhibiting the Trk receptor. For the attraction of nerves, the TrkB receptor is most important. However, TrkA and TrkC receptors also exist. Both of these receptors are upregulated in many cancer types (Meng, et al., 2019). Therefore, many Trk inhibitors designed target not only TrkB, but also TrkA and TrkC. Nowadays, already a few Trk inhibitors are FDA approved. Larotrectinib became the first approved inhibitor in 2018 (Scott, 2019).
These inhibitors are used to treat TRK-fusion positive cancers (Drilon, et al., 2018) . Normally, dimerization of the Trk receptor is required for its activation. In TRK-fusion positive cancers, the receptor becomes ligand independent. This results in constitutive signalling, leading to oncogenic effects. TRK fusions have been identified in a number of cancers including breast, colorectal and lung cancer (Vaishnavi, Le, & Doebele, 2015). Whether the use of a Trk inhibitor also influences tumour innervation has to my knowledge not been studied yet.

Discussion

This essay discusses how activation of β-receptors can influence tumour progression. Some of the processes discussed require activation of the β1-receptors specifically. In other processes the β2- or β3-receptor plays a role. In the study by Guo et al. (2013) it was for example found that the β2-receptor is responsible for the effects of NE on PNI in pancreatic cancer. This was found to be regulated via PKA, a protein that can also be activated by the β1-receptor. However, inhibition of the β1-receptor did not lead to a decrease in PNI. This is quite remarkable since Rains et al. (2017) found that the β1-receptor was upregulated in pancreatic cancer and NE has a higher affinity for the β1-receptor, compared to the β2-receptor. This raises the question why the β2-receptor and not the β1-receptor plays a role here. It is possible that the results of Rains et al. (2017) can not be translated to this study. This study did not determine the amount of β1-receptors and it can therefore not be concluded if they were actually present in these tissues. Another aspect that has not been discussed in this study is the β3-receptor. Rains et al. (2017) demonstrated that β3-receptors were also present in pancreatic cancer tissue. Besides this, they are able to stimulate PKA. It can therefore not be excluded that β3-receptors play a role in this process. This is also true for other studies discussed in this essay. A few examples are the studies by Sood et al. (2010) and Yang et al. (2009), which did not look at the influence of the β3-receptor. However, since the affinity of NE for the β3-receptor is much lower compared to the β1- or β2-receptor, it is more likely that the latter play a role in the effects on tumour progression. One study that did demonstrate the influence of the β3-receptor is the study on BNDF (Allen, et al., 2018). Here, it might be possible that the β3-receptor was higher expressed compared to the other β-receptors, something that again was not looked at. Overall, it can not be concluded why a specific β-receptor is important in a certain process. It might be the case that one receptor subtype is just higher expressed in a certain tissue. If this is true, this could mean that the processes discussed in this essay might be mediated via a different β-receptor in a different cancer type. For example, the effect of NE on BDNF could be mediated via the β1- or β2-receptor in cancer types other than pancreatic, since both of these receptors are able to increase cAMP. Future research could try to replicate the findings of Rains et al. (2017). This would lead to a better understanding of which β-receptor subtype is expressed in which cancer types.

NE influences cancer progression through a variety of processes. Inhibiting this signalling therefore reduces various aspects of cancer progression simultaneously. For this reason it also seems more favourable to use a β-blocker or denervate a tumour instead of using a Trk inhibitor. The TrkB receptor is only involved in the recruitment of nerves, while the β-receptor

is involved in all the processes discussed in this paper. During the recruitment of nerves, binding of NE to the β-receptor precedes the binding of BDNF to the TrkB receptor. By using a β-blocker you thus also inhibit the recruitment of nerves. However, it might also be possible that the TrkB receptor can become ligand-independent. TRK gene fusions that result in a constitutively activated Trk receptor have already been identified in various cancer types. These fusions might therefore also occur in nerves. If this is the case, the TrkB receptor can be activated without binding of BDNF, leading to increased innervation of a tumour. A β-blocker would not work in this situation. Whether TRK gene fusions influence tumoral innervation is something that could be studied in future research.

The results on β-blocker use are mixed. A downregulation of mesenchymal genes was found in one study, suggesting that EMT was inhibited. However, the meta-analyses discussed all conclude that β-blocker use has no beneficial effect on cancer progression. These analyses however, include results from different cancer types, from non-selective and selective β-blockers and also from different periods of β-blocker use. It might therefore be the case that β-blockers not work in every situation but do in some. A finding that supports this is the positive association found between β-blocker use and longer overall survival in melanoma, ovarian and pancreatic cancer but not in other cancer types. The benefits of β-blocker use were greatest in melanoma. The fact that all three β-receptors are most highly expressed in melanoma raises the question if there could be a link here. It might be possible that the amount of β-receptors in a tumour microenvironment influences the effect of a β-blocker. One limitation of these meta-analyses is that the results were based on only a few studies. Additional research in the effects of β-blocker use in melanoma, ovarian and pancreatic cancer can help to verify or refute the previous findings. Besides looking at these specific cancer types, future research could also focus on non-selective β-blockers. In the meta-analysis of Na et al. (2018) only two studies that specifically looked at a non-selective blocker were included. In the meta-analysis of Yap et al. (2018), only one was included. However, this essay discusses many processes that are dependent on the β2-receptor. Selective β-blockers only inhibit the β1-receptor and do not have an effect on β2-receptors. It could therefore be valuable to observe how only the use of a non-selective blocker affects tumour progression.

Sympathetic denervation has also been described as a potential part of cancer treatment. It was found that sympathetic denervation in a rat model of breast cancer reduced tumour growth. Whether this can be translated to humans or other cancer types remains to be explored. However, there are some limitations. Breast cancer is a more superficial cancer making it relatively easy to perform surgery. It might not be possible to reach a tumour located deeper inside the body. Also, when a tumour metastasizes it is often not known where all the

tumour cells are located and it is therefore not possible to remove the sympathetic nerves. Moreover, it has been demonstrated that a tumour can recruit sympathetic nerves. After removal it might be possible that the tumour recruits new nerves or even stimulates the formation of nerves from stem cells. Another problem that arises with sympathetic denervation is normal functioning of the organ. Sympathetic nerves regulate the normal functioning of organs and this might be impaired after surgical denervation. Not removing all sympathetic nerves allows the tumour to recruit them again or migrate towards them in the process of perineural invasion.

It is also possible to silence sympathetic nerves using an adeno-associated virus vector. With this method sympathetic nerves can be targeted very precisely. However, the problem of normal functioning still exists. New nerves can be recruited from the essential nerves that can not be removed. This would mean that a single injection is not sufficient and the therapy needs to be repeated to get lasting results. Moreover, the vector needs to be injected intratumorally. When a tumour is not superficially located or has metastasized, this would be difficult.

Another possibility of targeting the sympathetic pathway is to focus on proteins downstream of the β-receptor. Possible downstream proteins would include, STAT3, Src, FAK and MCP1. Src and FAK could potentially be targeted using a kinase inhibitor. However, as mentioned previously, this would mean that only one specific pathway is inhibited.

Conclusion

The effect of the tumour micro-environment on cancer progression is starting to be explored more and more. One component of this environment are sympathetic nerves. They release NE, which binds to β-receptors on tumour cells. It has been found that the amount of β-receptors in a tumour micro-environment is often higher compared to its surrounding healthy tissue. Moreover, it has been demonstrated that a high number of sympathetic nerves surrounding a tumour is associated with poor disease outcome. These findings suggest that the binding of NE to β-receptors plays a key role in cancer progression. This assay therefore aims to illustrate how this binding can influence cancer progression and if this process can be targeted in cancer treatment.

It was found that NE stimulates perineural invasion, angiogenesis, metastasis and immune suppression. It promotes angiogenesis by stimulating the release of pro-angiogenic factors and inducing a shift towards endothelial glycolysis. Metastasis is increased by upregulation of genes involved in the epithelial-to-mesenchymal transition, inducing resistance to anoikis and increasing invasiveness of tumour cells. Additionally, activation of β-receptors stimulates the recruitment of macrophages towards the tumour site and induces a shift towards the M2 phenotype. A last process influenced by NE is perineural invasion. Elevated levels of NE are associated with increased perineural invasion and decreased disease prognosis. Overall, all the findings demonstrate that binding of NE to β-receptors negatively influences cancer progression.

Since sympathetic stimulation is associated with poor disease outcome, it is emerging as a new target in cancer treatment. There are many aspects of this signalling that can be targeted. First of all, the source of NE can be inhibited by denervation of the tumour. Surgical denervation and genetic manipulation have been discussed as possible ways of reducing NE release. B-blockers do not have an effect on NE levels but inhibit the activity of the β-receptors. Trk inhibitors target a receptor even further in the pathway. Some questions remain for all of these techniques. Surgical denervation and genetic manipulation have only been tested in a rat model of breast cancer. Whether these can be translated to humans and other cancer types remains to be explored. The results of β-blocker use are mixed. Overall no beneficial effects have been found. However, analysis of cancer types separately have found positive results for melanoma, ovarian and pancreatic cancer. It therefore seems that β-blocker use might not be beneficial in all situations and cancer types. However, one big advantage of β-blockers are that they are already used nowadays to treat other conditions. This also the case for Trk inhibitors. A few Trk inhibitors have already been developed and approved by the FDA.

However, the effects of these inhibitors on tumour innervation have not been studied yet. Overall, it can be concluded that there is a lot of potential in targeting the sympathetic signalling pathway but more research is needed.

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