

# The Role of the Tumour Microenvironment on the development of Medulloblastoma

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*This review discusses the effects of the tumour Microenvironment on the development of tumour with a focus on Medulloblastoma. Furthermore it also expresses the importance of the tumour microenvironment on the Development of tumours.*



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## Abstract

A lot is already known about several genetic mutations that play a role in the nascence of cancer. However a lot is still unknown about the possibility of the involvement of microenvironmental factors in the development of cancer. This review therefore focuses on the factors in the tumour microenvironment which are already known and link these to Medulloblastoma, since a lot is still unknown about this specific type of cancer and since it still remains the most malignant type of tumour in infants and young children.

This review will focus on the role of the immune system; tumour associated macrophages, myeloid derived suppressor cells, epithelial-to-mesenchymal transition, cancer associated fibroblasts, hypoxia, oxidative stress, Wnt, SHH, Notch, BMP, TGF- $\beta$  and NMDAR.

All of the above mentioned factors are individually involved in the development of cancer and tumour progression all contributing to one of the multiple mechanisms of cancer metastasis and tumour progression.

Some of these factors overlap with the development of Medulloblastoma, especially some cells of the immune system, the Wnt and SHH pathways and TGF- $\beta$ .

This overlap emphasizes the importance of these factors and indicating a synergic effect of these factors. This is because the development of tumours never depends on one single factor.

Furthermore, these factors could in the future lead to specific targets for new therapeutic treatments of cancer.

**Key Words:** Medulloblastoma, tumour microenvironment, Tumour Associated Macrophages, Myeloid Derived Suppressor Cells, Epithelial-to-Mesenchymal Transition, Cancer Associated Fibroblasts, Hypoxia, ROS, SHH, Notch, Wnt, BMP, TGF- $\beta$ , NMDAR.

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## Introduction

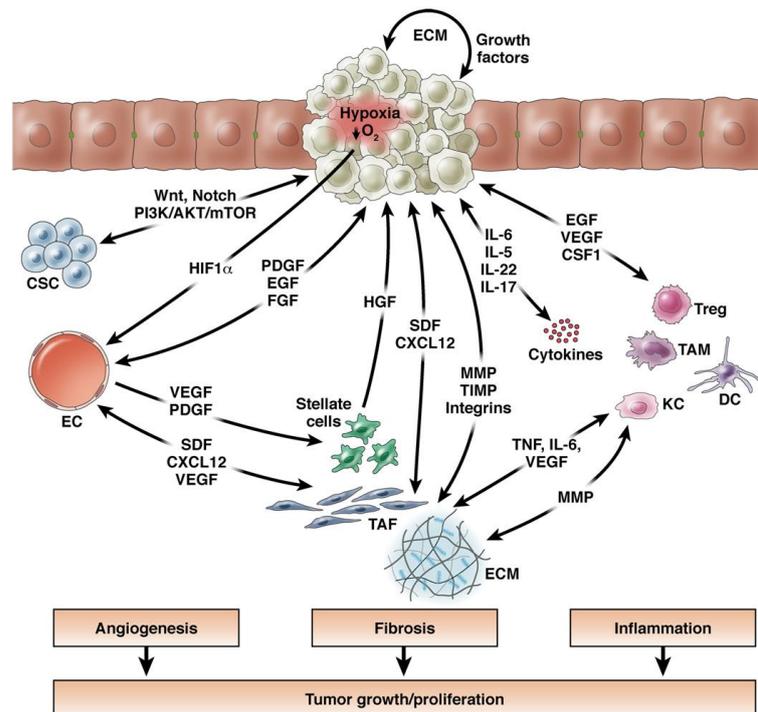
Brain tumours are a common type of cancer seen in young children. A good cure with 100% remission isn't available yet. Medulloblastoma is the most aggressive form of brain tumours in children<sup>1</sup> because it can use several growth factors to induce growth, proliferation, metastasis and the other hallmarks typical of cancer cells.<sup>2</sup>

A lot is known about several genetic mutations that can lead to cancer development especially about oncogenes and tumour suppressor genes like Myc and p53 respectively.<sup>3</sup> A single mutation in an oncogene can already lead to the development of cancer cells when there are already more mutations accumulated, since a mutation in an oncogene is dominant. Therefore only one extra mutation is needed. While on the other hand two mutations are needed in order to diminish the protective effect of a tumour suppressor gene in situations where several mutations are already accumulated, since mutations in tumour suppressor genes are recessive. Therefore, one mutation in a tumour suppressor gene isn't sufficient to establish the loss of tumour protecting function. Medulloblastoma is often induced by hereditary mutations<sup>4</sup> in tumour suppressor genes which make it even harder to treat this specific type of cancer. Furthermore, due to the mutations in the cancer cells in the core of the tumour (the so-called Cancer Stem Cells) reappearance of this type of cancer is often seen after treatment.<sup>5</sup> This is due to the fact that the cancer stem cells are multidrug resistance (which is one of the characteristics of CSCs). These CSCs have specific mutations that make them CSCs and therefore they are the most difficult to target.

However, mutations aren't the only factors contributing to the development of tumours. Like any other developing organisms there's a question about nurture and nature: Does something happen because it is set in someone's DNA? Or do environmental factors also play an important role in this process? Recently the focus of researches has shifted from the role of genetic mutations to the role of environmental factors and their influence on the development of cancer. This has even led to the so-called "seed and soil hypothesis".<sup>6</sup> This hypothesis states that a one must see a tumour as a complete organ instead of just a bunch of cells. It also says that the environment and the tumour interact with each other and that one could see a tumour as a seed and the environment as the soil: the seed cannot grow when the soil is polluted and of insufficient quality.<sup>7</sup>

This sheds a light on a completely new perspective of the development of cancer and possible new treatments for when one's able to make pollute the soil, the tumour won't be able to grow anymore.

Recently a lot of studies have therefore been published about several environmental factors that all contribute to the development of cancer in their own individual ways.<sup>8</sup>



**Figure 1 - Several cellular as well as soluble factors that each contribute to specific mechanisms of the development of tumour growth and progression**

It has become clear that there are a lot of environmental factors playing an important role in the development of tumours and that those factors are tumour specific therefore making it difficult to draw a complete picture of every type of cancer. However, there are several factors playing a role in the development of multiple types of cancers. (See fig. 1)<sup>9</sup>

This review will look at the cellular as well as the soluble factors in the tumour microenvironment that play a role in the existence as well as the development of tumours and where possible link these factors to the development of Medulloblastoma. (See table 1) As mentioned earlier, there are many factors playing a role or rather too many factors playing a role in the development of cancer.

Therefore this review will not go through all of them, but it will limit itself to the most frequently seen factors in multiple cancer progressions and to the most well-known factors. The cellular factors that will be discussed in this review are Tumour Associated Macrophages (TAMs), Myeloid Derived Suppressor Cells (MDSCs) the phenomenon of Epithelial-to-Mesenchymal Transition (EMT) and the Cancer Associated Fibroblasts (CAFs).

SHH, Notch, Wnt, BMP and TGF $\beta$  are the soluble factors what will be discussed in this review.

Furthermore I will also discuss the importance of other environmental factors like hypoxia, acidity and the role of oxidative stress on the existence and development of cancer.

Other factors that play also a role in the development of cancer are the extracellular matrix (ECM), growth factors (EGF, VEGF, FGF and HGF)<sup>10</sup> and several cytokines. (See fig. 1) However, a lot is already known and published about these factors; therefore these factors will not be discussed in this review.

**Table 1-An overview of the subjects to be discussed in this paper (in bold) and other factors contributing to the development of cancer.**

<b>Cellular Factors</b>	<b>Soluble Factors</b>
<b>Tumor Associated Macrophages</b>	<b>Hypoxia</b>
<b>Myeloid Derived Suppressor Cells</b>	<b>Oxidative Stress</b>
<b>Epithelial-Mesenchymal Transition</b>	<b>Wnt, SHH, Notch</b>
<b>Cancer Associated Fibroblasts</b>	<b>TGF-<math>\beta</math>, BMP</b>
	<b>NMDAR</b>
	ECM
	EGF, VEGF, FGF, HGF
	Cytokines

## Tumour microenvironment

There are a lot of factors in the microenvironment of a tumour that play a role in the development, invasion and metastasis of tumours. In this chapter we will discuss the most important and well-known factors that contribute to the growth of a tumour. We will discuss cellular factors as well as soluble factors.

### Cellular factors

During tumour development and progression a lot of different cells play an important role in the contribution of the optimisation of the tumour microenvironment in order to prepare the tumour for invasion a metastasis. Some of these cells appear at the tumour site with the best intentions to destroy the tumour, but unfortunately their actions show an opposite effect on the tumour.

### Immune system

The immune system plays an important role in the development of cancer. There are several factors contributing to the genesis of cancer but this section will focus on the role of IL-8, TAMs and MDSCs

### Tumor Associated Macrophages

Tumour associated macrophages (or TAMs) seem to be crucial in tumour progression but so does the rest of the immune system with chronic inflammation being the main malefactor<sup>11</sup>. It is known that pro-inflammatory cytokines lead to increased motility of neighbouring cells<sup>12</sup>, which, in turn, leads to the creation of more space for the tumour to grow. This is due to their activation but it's also partly due to the cell adhesion molecules which are being cleaved.<sup>13</sup> This activation can occur through inflammation or an infection but it can also occur in combination with oncogene activation leading to a cancer-related inflammation which will in its turn lead to tumour progression (see fig. 2)<sup>14</sup>. It must be said that cancer cells themselves are able to secrete cytokines and chemokines but these can also be secreted by cells of the immune system, fibroblasts and endothelial cells as a response to stress established by growing cancer cells<sup>15</sup> and to increase the fertility of the so-called soil. One of those chemokines that can be secreted by cancer cells undergoing Epithelial-to-Mesenchymal Transition (EMT) is Interleukin 8 (IL-8).<sup>16</sup> The cancer cell secrete IL-8 in order to maintain the EMT (which will be discussed further on) which will lead to metastasis. Also, IL-8 shows a link between tumour stemness, which is important for proliferation and self-renewal of the cancer cells.<sup>17</sup> Furthermore, it has been shown that enhanced secretion of IL-8 by cancer cells serves to enhance the recruiting of neutrophils.<sup>18</sup> These neutrophils in turn serve to stimulate protumorigenic and prometastatic functions which lead to tumour progression and metastasis.<sup>19</sup>

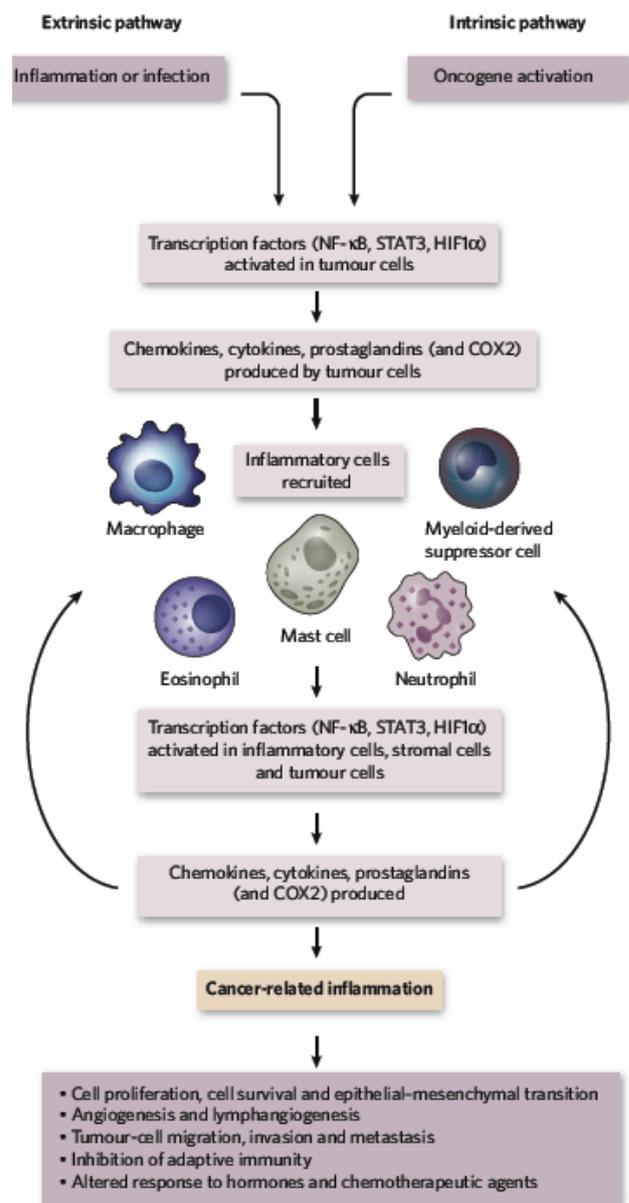


Figure 2- An interplay between intrinsic factors (inflammation) and extrinsic factors (oncogene activation)

So it's clear that the immune system plays a role in the development of cancer, but TAMs in particular have a specific part in this.

TAMs are characterized as one of the driving forces of angiogenesis, together with other leukocytes that have been recruited to the tumour site, which is a crucial step in metastasis.<sup>20</sup> The TAM-induced angiogenesis is enhanced by a VEGF-independent pathway by the secretion of pro-angiogenic factors.<sup>21</sup> TAMs have a pro-inflammatory effect and it induces tumorigenic functions as growth, remodelling of tissues (e.g. EMT), angiogenesis<sup>22</sup> and it suppresses the adaptive immune system so that the tumour can escape from the cytotoxic effects of the immune system.<sup>23</sup> Furthermore, it has been shown that there is a correlation between the density of macrophages present at the tumour site and a poor prognosis for the patients.<sup>24</sup>

### Myeloid Derived Suppressor Cells

Other cells that find their origin in the myeloid lineage and play a role in the development of cancer are the so-called Myeloid Derived Suppressor Cells (MDSCs). MDSCs are actually immature immune cells which accumulate in the tumour niche of the primary tumour.<sup>25</sup> MDSCs can express C11b and Gr-1 antigens<sup>26</sup> and are therefore able to suppress the T-cell activation by secretion of the immune suppressing IL-12.<sup>27</sup> Therefore MDSCs play an important role for the primary tumour in their hallmark of the suppression of the adaptive immune system.<sup>28</sup> Other functions of MDSCs are the promotion of angiogenesis through MMP9-production.<sup>29</sup> These secreted factors work in a paracrine signalling loop which leads to extra stimulation of cancer cells.<sup>30</sup>

Of course there are a lot of other small factors within the immune system that all contribute to the development and progression of cancer, like IL-6 which seems to mediate the fibroblast activation or T-lymphocytes which play a role in the antitumor response by the immune system.<sup>31</sup> Nevertheless, the above mentioned cells (TAMs and MDSCs) are the main driving forces of the inflammation-driven cancer progression.

### Epithelial-to-Mesenchymal Transition

Another phenomenon that is characteristic of tumour invasion and metastasis is called Epithelial-Mesenchymal Transition (EMT). EMT refers to a change in the composition of the tumour surface. EMT is not only present in tumorigenesis but it's also seen in embryonic development and wound healing, since it is a process that leads to migration of cells in order for them to continue executing their functions elsewhere.<sup>32</sup> The tumour surface is characterised by a layer of epithelial cells which are all dependent on each other and are immobile.<sup>33</sup> However, in cases of metastasis the epithelial cells change to mesenchymal cells through EMT.

<sup>34</sup> These new mesenchymal cells have obtained mobility and therefore they can move through the extracellular matrix (ECM) and therefore enhance metastasis.<sup>35</sup> EMT is characterised by a downregulation of the adhesion molecule Ecadherin<sup>36</sup> and an up-regulation of the anchoring molecule Vimentin<sup>37</sup> in order to keep the mesenchymal cells together when

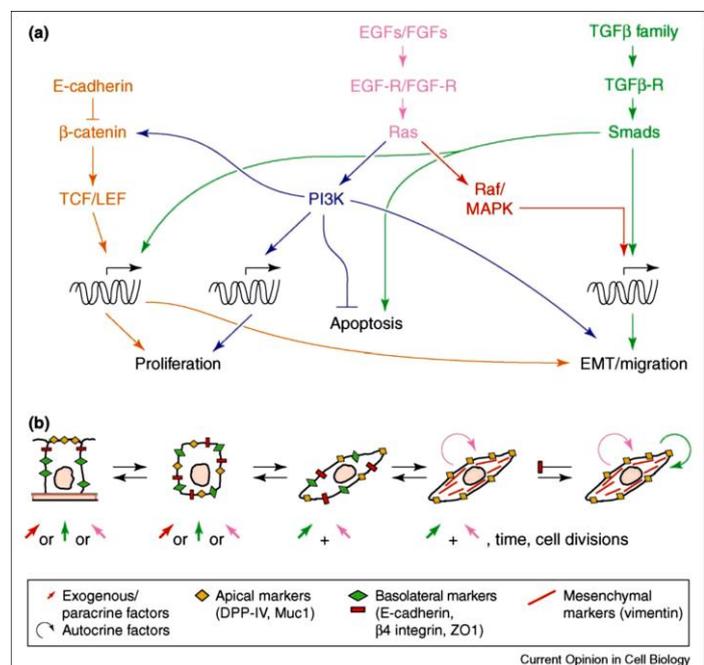


Figure 3 - The process of EMT with (a) showing the mechanisms underlying EMT and (b) showing the remodelling of cells (with the 1st picture being an epithelial cell and picture 5 being a mesenchymal cell)

invading the ECM. (See fig. 3)<sup>38</sup> Another consequence of EMT is the induction of stemness of the tumour-initiating cells.<sup>39</sup> Therefore EMT leads to invasion and progression of the tumour growth, but it also leads to more cancer stem cells (CSCs) to maintain the primary tumour.

## Cancer Associated Fibroblasts

Then there are also Cancer Associated Fibroblasts (CAFs) that play a role in the development of cancer. CAFs are associated with bad prognosis in cancer patients and can appear as a side product of EMT.<sup>40</sup> CAFs are found to be important cells during the migration process during EMT but also during TAMs recruitment.<sup>41</sup> CAFs can produce Fibronectin and Hyaluronan which play an important role in migration, cell adhesion and growth.<sup>42</sup>

Hyaluronan is a section of the ECM and its function depends on its concentration: when it's highly expressed it enhances an anti-inflammatory effect, whilst it enhances proliferation and inflammation when it's low expressed.<sup>43</sup> Therefore it enhances a protective effect in naked mole rats where Hyaluronan is overexpressed<sup>44</sup> but it enhances metastasis in tumours because CAFs regulate the expression of Hyaluronan in such a way that it's only limited produced and therefore promoting proliferation.<sup>45</sup> Furthermore, due to a high concentration in naked mole rats and their high sensitivity to this substance, naked mole rats exhibit a much higher elongation than their associated Rodents like the mouse and the rat and don't develop cancer.<sup>46</sup> Therefore this substance is a rather interesting factor contributing to cancer research.

CAFs can also promote stemness of the CSCs<sup>47</sup> but in the end the main function of CAFs is to enhance EMT and promote invasion of the tumour and to eventually bring nontumorigenic cells into a tumorigenic state in order to metastasize to neighbouring tissues and make them malignant.<sup>48</sup> Furthermore, CAFs have many different functions. They can produce growth factors and cytokines<sup>49</sup> - all being tumour specific and can therefore activate the immune system and promote angiogenesis.<sup>50</sup>

## Soluble factors

Next to cellular factors, there are also soluble factors involved in the development and progression of tumours. These include signals secreted by other cells that promote several hallmarks of cancer.

## Hypoxia

Hypoxia occurs when there isn't enough oxygen present in the environment in order for the cells to function. The oxygen levels then fluctuate between 0,1 % and 3,0 %.<sup>51</sup> This fluctuation is mediated by factors produced by the cancer cells called Hypoxia Inducible Factors (HIF) and they exist of two alpha subunits and a beta subunit.<sup>52</sup> Under normal oxygen levels the alpha subunits are targeted for degradation and will therefore have no specific function.<sup>53</sup> However, under hypoxic conditions the dimerization of the alpha subunits with the beta subunit are being stabilised and the HIF transcription factor can do its job. It binds to HIF response elements (HREs) which leads to angiogenesis through VEGF, invasion (through EMT)<sup>54</sup> and differentiation of cancer cells in combination with Wnt.<sup>55</sup> Hypoxia itself can therefore lead to the malignancy of tumours (promoting invasion and metastasis) and makes it therefore difficult to treat them.<sup>56</sup>

## Oxidative Stress

Oxidative stress is another factor that promotes invasion and metastasis, especially through EMT.<sup>57</sup> Oxidative stress is due to Reactive Oxygen Species (ROS) or Nitric Oxygen Species (NOS) which are produced in the tumour stroma by macrophages and by mitochondria of cancer cells.<sup>58</sup> However, ROS can also be produced by a lack of sufficient oxygen supply<sup>59</sup> or oxidation of NADPH.<sup>60</sup> CAFs are recruited to the site of the tumour as a response to oxidative stress, which, in turn, leads to migration, invasion and metastasis.<sup>61</sup> Furthermore, oxidative stress can lead to DNA damage and therefore can promote the onset of cancer.<sup>62</sup> Therefore oxidative stress has a dual function since it's required for the maintenance of cells when produced in limited amounts. However, when it's being overproduced, it can enhance tumour progression and onset.<sup>63</sup>

## Wnt

Other factors which play an important role in the development of cancer, but also play a role in embryonic development are Wnt, SHH, Notch and BMP. They play a role in the differentiation of developing stem cells during embryonic development, but also during the rest of one's life in the renewal of the intestinal and pulmonary epithelium.<sup>64</sup>

Wnt is a ligand that binds to the Frizzled receptor and the LRP5/6 co-receptor which leads to accumulation of  $\beta$ -catenin.<sup>65</sup> This accumulation of  $\beta$ -catenin stimulates cell adhesion which is needed for the maintenance of the epithelium but also for the development of mesoderm and the gastrulation in embryonic development.<sup>66</sup>

However, mutations in the Wnt-frizzled pathway can lead to the loss of the APC tumour suppressor genes. When the APC tumour suppressor genes are lost, the Wnt-signaling pathway also loses its normal regulation which leads to excessive and uncontrollable growth of epithelium or other cells.<sup>67</sup>

## SHH

Sonic Hedgehog (SHH) is the most well-known isoform of the Hedgehog family, which exist further of Indian hedgehog and Desert hedgehog.<sup>68</sup> SHH can bind to the Patched-receptor. As a result of this bond, Smoothed, which is a membrane-bound protein, is being repressed and Gli transcription factors are being activated. Activation of Gli-proteins can lead to the activation of Wnt-signalling pathway, which, in turn, leads to lethality of healthy cells and tumour progression.<sup>69</sup>

## Notch

Then there's also the Notch pathway which is involved in the onset of cancer. The Notch pathway consists of four receptors called Notch-1, Notch-2, Notch-3 and Notch-4. There are several ligands that can bind to the Notch receptors, namely: Jagged-1, Jagged-2, Delta-1, Delta-3 and Delta-4.<sup>70</sup> Binding of one of these ligands to receptors of the Notch family induces activation of genes that were repressed before.<sup>71</sup>

However, the activation of the Notch-1 pathway has multiple and opposite effects on the onset of cancer. It has been said that mutations in Notch-1 might play an oncogenic role in the development of T-cell Acute Leukaemia (TALL) while studies have also shown a tumour suppressing effect in the onset of Chronic Myelomonocytic Leukaemia (CMML).<sup>72</sup> Albeit, the function of Notch-1, during the onset of cancers of the hematopoietic system, depends on the cell type.<sup>73</sup> Unfortunately, this is also true for the solid tumours. Studies have shown that Notch-1 can have an oncogenic as well as a tumour suppressing effect on the onset of cancer.<sup>74</sup> Since it's involved in cell fate decisions and is needed to decide whether or not a cell is going to proliferate with a mutation or whether it's going to induce apoptosis to avoid proliferation with mutations.

## TGF- $\beta$ and BMP

Another factor that is well-characterized in the development of cancer is Transforming Growth Factor- $\beta$  (TGF- $\beta$ ). TGF- $\beta$  is an interesting type of cytokine since it serves as a tumour suppressor in healthy epithelial cells and during early tumour growth, whilst it serves as an oncogenic factor in later stages of tumour development.<sup>75</sup>

The TGF- $\beta$  signalling pathway is mediated by a Smad-dependent as well as a Smad-independent pathway and is involved in many mechanisms related to cancer. There are three types of isoforms of TGF- $\beta$ , namely: TGF- $\beta$ 1, TGF- $\beta$ 2 and TGF- $\beta$ 3. Since there are three isoforms, it is obvious that there are also multiple receptors available to which TGF- $\beta$  can bind. There are 12 receptors in total to which TGF- $\beta$  can bind. There are seven type I receptors; the so-called ALKs1 until 7. Then there are the five type II receptors: ActR-IIa, ActR-IIb, BMPRII, AMHRII and T $\beta$ RII.<sup>76</sup> Then there is also a type III receptor, however, this receptor doesn't establish a direct effect on itself, but it helps the TGF- $\beta$  ligands to better bind to their allied receptors; the type I and type II receptors.<sup>77</sup>

TGF- $\beta$  can establish the phenomenon of EMT and thus result in tumour progression via autocrine or paracrine stimulation.<sup>78</sup> TGF- $\beta$  therefore promotes cell motility and invasion.<sup>79</sup>

Furthermore TGF- $\beta$  can also stimulate an initiation which is dependent on ROS-concentrations and lead to a switch of fibroblasts into myofibroblasts during Mesenchymal-mesenchymal transition (MMT). This MMT leads, in turn, to immune suppression and evasion, angiogenesis<sup>80</sup> and eventually to metastasis and invasion of the tumour.<sup>81</sup>

Furthermore, binding of TGF- $\beta$  to its receptor results in degradation of RhoA, this is responsible for the polarity and junctional stability of cells. When RhoA is degraded the epithelial cells lose their polarity which allows EMT to occur.<sup>82</sup> Also, partly due to this phenomenon, TGF- $\beta$  enhances the ability of the tumour to escape from the immune system by inducing apoptosis or survival through EMT.<sup>83</sup>

Due to its influence on apoptosis, TGF- $\beta$  is also involved in the regulation of the normal cell cycle. This in order to maintain the homeostasis of organs to prevent them from overgrowing itself<sup>84</sup>, therefore it is said that TGF- $\beta$  has a tumour suppressor function (in healthy cells as well as in cancer cells) on the development of cancer.<sup>85</sup>

Therefore, the main function of TGF- $\beta$  is to keep the epithelial proliferation limited and therefore halt the premalignant growth of a tumour (see fig. 4)<sup>86</sup>. Nevertheless, these effects are only present in situations where the tumour itself is under oncogenic stress (and needs to be defended against this) or tissue injury, which, more often than not, result in EMT.<sup>87</sup>

Bone Morphogenetic Proteins (BMPs) are also involved in the development of cancer. BMPs belong to the TGF- $\beta$  superfamily (which will be discussed further on) and are involved in the formation of bones. The proteins bind to the BMP-receptors called BMPR-1 and BMPR-2, which are both Serine-threonine kinase receptors. Therefore, binding to BMPR-2 leads to phosphorylation of BMPR-1, which will in turn, lead to activation and nuclear localization of the epithelium.<sup>88</sup>

Mutations in the BMP signalling pathway can lead to excessive activation of the Wnt signalling pathway, resulting in tumour progression.<sup>89</sup>

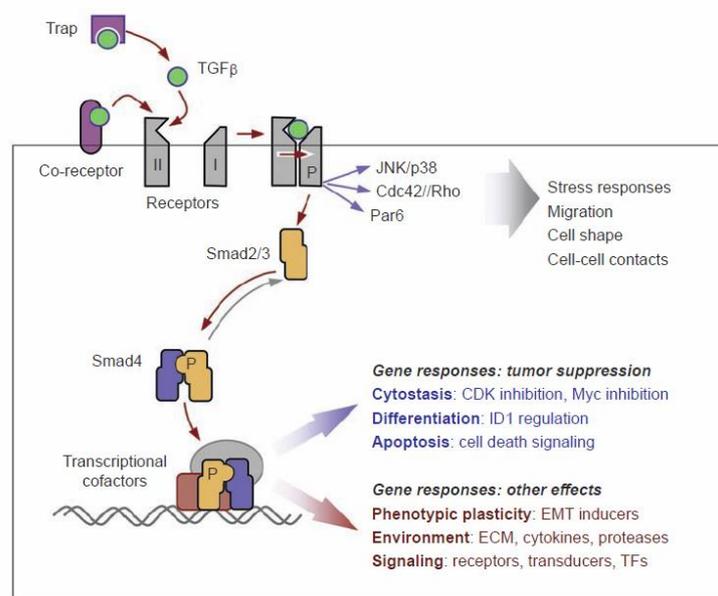


Figure 4 - The mechanisms of TGF- $\beta$  signalling pathway and its effects on tumour progression and invasion

## NMDAR

Another interesting factor that specifically plays a role in the development of several brain tumour is the N-methyl-D-aspartate receptor or NMDAR. This receptor is involved in the learning process as well as memory formation and neuron maturation since it serves as a prevailing factor in the process of neuronal plasticity.<sup>90</sup>

However, this pathway is also used in the development of cancer. The ligand that binds to this receptor is the amino acid Glutamate. It has been shown that tumour cells secrete glutamate which therefore means that tumour cells stimulate their own growth through paracrine or autocrine stimulation.<sup>91</sup>

Tumour cells start secreting this amino acid when the interstitial fluid pressure (IFP) in the tumour stroma is elevated to a level in which invasion is advised. The growing tumour builds up this pressure because it grows in a small space. When a tumour becomes too large, because of continuously growing and differentiating cancer stem cells, a pressure is being built inside the stroma. When this IFP is too much elevated, an interstitial flow occurs, since the IFP of surrounding tissues is lower than that of the tumour.<sup>92</sup> This flow exists of a diffusion of substances from the tumour stroma towards the surrounding tissues. Due to this phenomenon, cancer cells start to secrete glutamate and thereby activating the NMDAR signalling pathway, which prepares the tumour for invasion and metastasis in order to lower the IFP.<sup>93</sup> Therefore, activation of the NMDAR signalling pathway leads to proliferation of tumour cells as well as invasion of surrounding tissues by the tumour cells.<sup>94</sup> After binding of glutamate to the NMDAR the CAMK/MEK-MAPK pathway is being activated. This, in turn, activates the transcription factor Creb which is associated with the Creb Binding Protein (CBP). This protein activates pro-invasive genes to prepare the cell for proliferation and invasion (see fig. 5)<sup>95</sup>. This highlights the role of glutamate and NMDAR in metastasis of brain tumours.

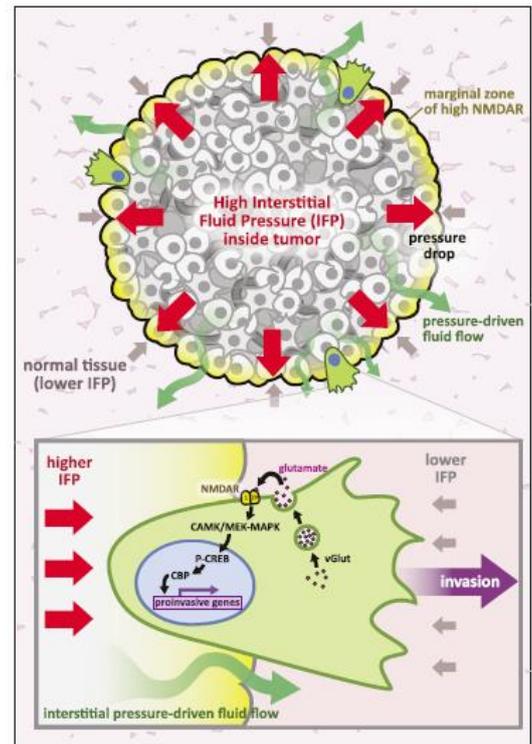


Figure 5 - The regulation of glutamate secretion by elevated IFP

## Discussion

This review was meant to shed some light on an upcoming interest of the role of the tumour microenvironment during not only the onset and development of cancer, but also during tumour progression and metastasis. All of the above was about cancer in general, but this section will serve as a comparative section to investigate which environmental factors of the above mentioned factors also play an important role in the development of Medulloblastoma.

First of all, it must be said that environmental factors are tumour specific and that not every environmental factor is the same in every type of tumour. Therefore it is not unlikely that the environmental factors mentioned earlier above aren't applicable to Medulloblastoma or any other type of cancer for that matter.

Nevertheless, the development of Medulloblastoma is also dependent on environmental factors and it studies show that there are some overlapping factors between Medulloblastoma and the factors mentioned earlier in cancer in general.

Studies have shown that there is a specific role put aside for the immune system in the development of Medulloblastoma. The adaptive immune system of patients diagnosed with Medulloblastoma is being suppressed, resulting in a dysfunction of T-lymphocyte activity.<sup>96</sup> Furthermore, due to this dysfunction, development of treatment against tumour specific targets for T-lymphocytes is limited. Nevertheless, it has also been shown that stimulation of the innate immune system, specifically elevation of the concentration of Natural Killer cells (NK cells) counters tumour progression and invasion.<sup>97</sup> NK cells have the ability to lyse medulloblastoma cells resulting in a decrease in tumour cells. Tumour progression results in a down regulation of HLA class 1 molecules and therefore its associated Natural Killer cell inhibitory receptors as well. This leads to a higher sensitivity of the tumour to NK cells and the NK mediated cytotoxicity.<sup>98</sup>

The proteins to which NK cells bind on the surface of the tumour cells are Major histocompatibility complex class I-related chain A (MICA) and UL16 binding protein 2 (ULBP-2). These proteins bind to the NKG2D receptors present on the cellular surface of the Natural Killer cells. This binding of the MICA and ULBP-2 proteins to the NKG2D receptors leads to activation of the Natural Killer cells and thus promoting lyses of the tumour cells.<sup>99</sup> The cytotoxicity of the NK cells doesn't only affect the metastasized tumour cells, but the cells of the primary tumour as well, making NK cell immunotherapy a plausible treatment for patients of Medulloblastoma.

Another interesting aspect of the immune system that is involved in the development of Medulloblastoma is the chemokine receptor CXCR4. This specific receptor is associated with proliferation and migration during cancer metastasis.<sup>100</sup> CXCR4 stimulates a GF-mediated pathway through PDGFR activation.<sup>101</sup> CXCR4 binds to CXCL12 and dimerizes after this binding and, in turn, is being phosphorylated. Phosphorylation of this receptor leads to induction of internalisation and lysosomal degradation.<sup>102</sup> This will eventually lead to signal termination and enhances cancer aggression.<sup>103</sup>

Unfortunately, there isn't much known about the influence of the rest of the above mentioned factors on the development of Medulloblastoma. Therefore we can conclude that either those factors simply aren't such important factors in the development of Medulloblastoma or not even involved in the development of Medulloblastoma. Another option of course is that it is still unclear if these factors play a crucial role during development of this specific type of cancer.

However, one particular section of this review does play an important role in this development since a lot is already known about its involvement in Medulloblastoma, namely the Wnt and SHH signalling pathways. Most Medulloblastoma cells either do not show signs of activity in the Wnt and SHH signalling pathway or show a disrupted Wnt and SHH signalling pathway.<sup>104</sup> That these factors play a crucial role in the development of Medulloblastoma becomes clear when realizing that Medulloblastoma can be divided in four subgroups of which two of them are named "Wnt-subgroup" and "SHH-subgroup".<sup>105</sup> The other two subgroups are simply called "group 3" and "group 4" and their classification is based upon their gene expression.

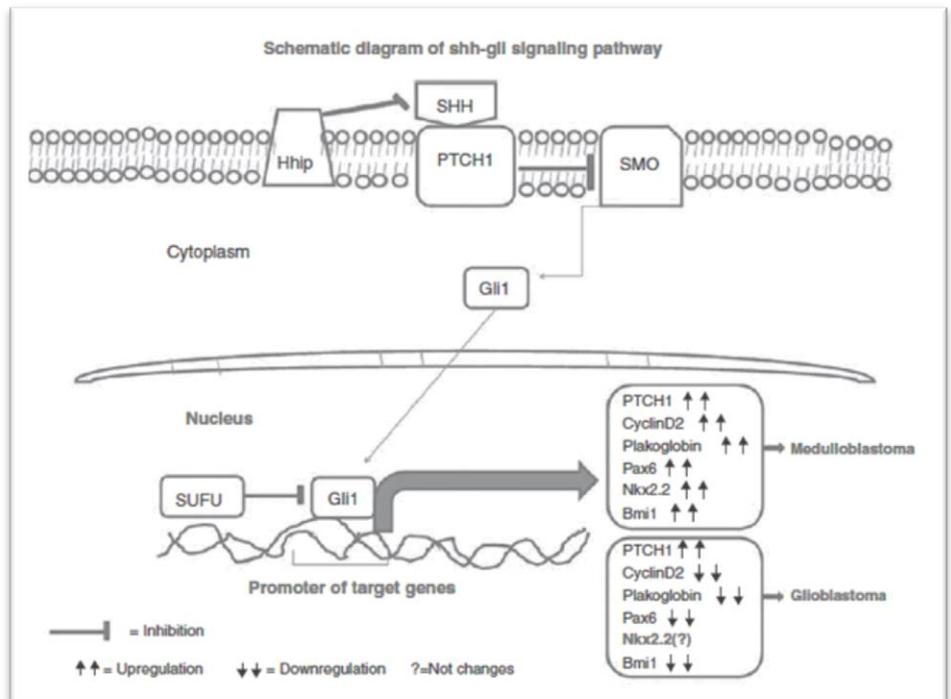
Nevertheless, as their name already gives away, the Wnt-subgroup and SHH-subgroup are classified on behalf of their genesis through mutations in either the Wnt signalling pathway or the SHH signalling pathway respectively. An example of a disruptive pathway is when the pathway is being activated while it shouldn't be activated.<sup>106</sup>

Tumours from the Wnt subgroup are often originated from progenitor cells in the embryonic dorsal brainstem. Whereas tumours from the SHH subgroup most likely find their origins in committed Granule Neuron Precursors (GNPs) of the cerebellum.<sup>107</sup>

The disruptive activation of the Wnt signalling pathway of patients belonging to the Wnt subgroup is often due to mutations in CTNNB1 (encoding  $\beta$ -catenin), APC, and AXIN1.<sup>108</sup> Uncontrollable expression of Wnt is associated with abnormal cell proliferation which leads to tumour progression.

Furthermore, the role of the SHH signalling pathway on the genesis of Medulloblastoma is better understood. An activated SHH signalling pathway eventually leads to suppression of Smoothed, which, in turn, activates the Gli transcription factors Gli1, Gli2 and Gli3.<sup>109</sup> Gli1 in particular plays an important role in the onset of Medulloblastoma. When Gli1 is being activated, it, in turn, stimulates transcription of target genes resulting in cancer development. (See fig. 6)<sup>110</sup>

Albeit, several micro-environmental factors in the development of Medulloblastoma are similar of those present in other types of cancer, suggesting an important role of these factors in the development of cancer. Even so, since these factors overlap each other in the genesis several types of cancer, they make an interesting new target for the treatment of cancer.



Figuur 6 – Overview of the SHH-Gli1 signalling pathway and their effects on the nascence of brain tumours.

## Conclusion

This section contains some concluding remarks concerning the role of the microenvironment on the development of cancer and tumour progression. As we have seen in this review there are several microenvironmental factors playing a role in the genesis of cancer and all being tumour and location specific. But nevertheless, they are important contributing factors to the progression of cancer. As been shown in this review, there are several different kinds of microenvironmental factors important in the nascence of carcinoma. These factors range from cellular factors to soluble factors. In this review we've also discussed some environmental factors such as hypoxia and oxidative stress. Hypoxia leads through VEGF and EMT to angiogenesis and invasion of the tumour. This of course leads to progression of the tumour.

On the contrary, oxidative stress can cause tumour progression mainly through EMT alone. Furthermore it can also cause DNA damage which leads to stimulation of the onset of cancer. Both of these consequences lead to tumour progression and invasion, making the tumour more aggressive and more difficult to treat.

One of the cellular factors that plays a role in the development of cancer is the cells of the immune system, with a particular role set aside for tumour associated macrophages (TAMs) and the chemokine IL-8. Both of these factors result in invasion and tumour progression; however both have their own ways in doing so. IL-8 plays a role in EMT, which as we've already seen, leads to metastasis. TAMs on the other hand promote metastasis and tumour invasion through its ability to promote angiogenesis.

Other cells that play an important role concerning the immune system and the development of cancer are the MDSCs. These cells suppress the adaptive immune system and promote angiogenesis. The phenomenon of epithelial-to-mesenchymal transition also plays a crucial role in the tumour invasion and progression, since it stimulates the mobility of cancer cells and induces the self-renewal of the cancer stem cells.

Cancer associated fibroblasts also contribute to the progression of cancer due to their ability to produce several factors including Hyaluronan to promote cell adhesion, migration and cell growth. Hyaluronan contributes to a longer life in naked mole rats and protect them from developing cancer. It is therefore an interesting factor for therapeutic treatments. Scientists believe that this might be a potential new medicine for cancer patients and are already experimenting with this substance on other rodents.<sup>111</sup>

Then there are several soluble factors involved as well. Wnt, SHH, Notch and BMP seem to play crucial roles in the development of several cancers. Although they all work through a different pathway, they all seem to result in tumour progression. However, some functions of these factors aren't completely clear yet, since they also seem to result in tumour suppressing functions. TGF- $\beta$  is another important factor in the genesis of cancer. This factor contributes to tumour progression and invasion in multiple ways. It can exhibit EMT as well as MMT (mesenchymal-mesenchymal transition) with both of these resulting in invasion, immune suppression, angiogenesis and thus resulting in metastasis.

Another interesting factor is NMDAR, which is involved in several brain tumours. NMDAR can induce, by binding of Glutamate, invasive and metastatic mechanism in order to promote tumour progression.

As we have now seen, several factors are seen in multiple types of cancer, emphasizing the importance of these factors in the development of cancer in general. This insight could lead to more specific targets of the tumour microenvironment for new therapeutic cancer treatments.

Of course, it must be said that it is not just one factor that induces cancer, but that several factors play a role in the progression of one specific type of cancer. This indicates that several factors together would have a synergic effect on the progression of carcinoma. Noting of course that together with these environmental factors, there are often also several genetic mutations that underlie the genesis of cancer.

Therefore it would be wise to tackle every environmental factor that is involved in a cancer. This might not tackle the entire tumour, but, to speak in terms of the “seed-and-soil”, when one makes the soil infertile by tackling all the environmental factors that are involved, the seed won’t be able to grow anymore. Even so, when the seed or the tumour isn’t able to grow and proliferate, the tumour might diminish. Of course it must be investigated first whether or not tackling of the environmental factors have negative effects on the surrounding healthy tissue (or any other cells or functions for that matter). Furthermore, there is still a lot unclear about several factors. Although we’ve already become a long way in discovering the role of factors in the tumour microenvironment, we’ve probably only reached the tip of the iceberg yet, therefore a lot of further research is needed to get a complete picture of all the factors in the tumour microenvironment.

Of course there are other external environmental factors that seem most obvious to mention in the development of cancer, like smoking<sup>112</sup> and obesity<sup>113</sup> which weren’t discussed in this review. These factors are of course also important since they also pollute the soil, but then in a positive manner for the tumour. However, these factors are just precursors of the mechanisms occurring internally like inflammation and these in vivo mechanisms are discussed in this review.

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