The role of secreted cellular factors after DNA damage in the development of senescence and the treatment of cancer

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Abstract

According to the World Health Organization (WHO), cancer is the leading cause of death worldwide. When DNA damage is not repaired, this will lead to lesions which can cause cancer. The DNA damage response (DDR) is a complex mechanism which includes DNA repair systems and checkpoints which detect the lesions. If a lesion is detected, than this mechanism will secrete signals which activates DNA repair. An important mechanism to permanently prevent proliferation in damaged cells is called cellular senescence. A major characteristic of cancer is that these cells proliferate unrestrained. If a therapeutic agent could induce cellular senescence in tumor cells than this could potentially be used to treat cancer. Furthermore, the microenvironment of tumors could be a good target for a treatment, in order to prevent tumor metastasis and tumor growth. Hereby, a successful strategy is to target non-tumor cells and the factors they secrete in the microenvironment of the tumor. Cellular senescence arise by the critical minimal length of the telomeres. 90% of the tumor cell have a telomerase activity which increases risk for developing cancer. Another option to treat cancer could be targeting of the telomerase. Understanding the role of factors secreted by cells after DNA damage, the development of cellular senescence and targeting tumor telomerase are topics which have potential for curing cancer.

In this thesis, I will investigate the role of factors secreted by cells after DNA damage and the development of cellular senescence, induction of which may be a potential therapeutic target. Therefore the research question of this thesis is: Do factors secreted by cells after DNA damage and the development of cellular senescence potentially lead to a cancer treatment?

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1. Introduction

According to the World Health Organization (WHO) cancer is the leading cause of death worldwide. Millions of people are diagnosed with cancer and most of them eventually die of this disease. The uncontrolled cell growth and the capacity to metastasize are the two essential characteristics of cancer (Ma, Yu 2006). The uncontrolled cell growth in various body cells leads to over hundred types of cancer (Cooper 2000). Each type is unique and, therefore, each type of cancer needs specific diagnosis and treatment (Sminkey 2015). There are many risks that cause cancer, for example smoking of tobacco. If people around the world prevent the use of tobacco, cancer could be reduced with 30%. Tobacco is one of the major offenders of cancer, by its ability for damaging the DNA (Hoeijmakers 2009). DNA is the most important carrier of our genetic material. Human body cells can loss their function, because of the mutations in DNA. There exist a mechanism which detect lesions in the DNA, this mechanism is called DNA damage response (DDR). The DDR has various functions in the cellular response. After the lesion is detected by the DDR it gives signals which activate the repair system. Beside the detection and repair of the lesions, DDR has also a role in cell cycle arrest and if necessary apoptosis. In addition, upon DNA damage, cells secrete signals for the communication between cells. A better understanding of the signaling of tumor cells may lead to the development of a new treatment for cancer. As mentioned above, tumor cells have a uncontrolled cell growth. A possible way to stop cell division is to induce apoptosis or arrest the cell growth in these tumor cells. Tumor cells loses their function of apoptosis, allowing these cells to divide and accumulate DNA damage. If there is a mechanism or drug that could induce apoptosis in these cells it could be a treatment to cure cancer. A known mechanism which prevents proliferation in tumor cells is called cellular senescence (Weintraub 2016). This mechanism induces irreversible growth arrest. Inducing this mechanism in tumor cells will lead to decreased tumor development. The great diversity of cancer types leads to an unique treatment in each type of cancer. It is necessary to develop new cancer treatments, because cancer is worldwide the leading cause of death.

Therefore, the research question for this thesis to answer is: Do factors secreted by cells after DNA damage and the development of cellular senescence potentially lead to a cancer treatment?

2. Substances that cause DNA damage

There are various factors that cause DNA damage, which can be divided into two different processes; endogenous and exogenous factors. DNA replication and oxidative lesions are examples of endogenous processes. Each day, thousands of lesions arise in the human genome, if these lesions are repaired incorrectly or are not repaired at all then these lesions lead to mutations which could cause cancer (Jackson, Bartek 2009). Another endogenous process which leads to DNA damage is oxidative stress. The main cause of oxidative stress is the production of reactive nitrogen/oxygen species (RNS/ROS) or the weakened capacity of anti-oxidants (Fan et al. 2016). This is one of the most important cause that lead to cancer onset and cancer progression, because it has the ability to change the gene expression, apoptosis and cell proliferation (Mileo, Miccadei 2016). The factors that cause DNA damage from exogenous processes are for example ultraviolet (UV) light. UV light is a major pervasive agent that causes DNA damage, this can lead to several types of DNA lesions (Gerlitz 2010). The most dangerous part of the UV spectrum is absorbed by the ozone layer, but even then the sunlight can induce 100.000 lesions, in the body cell in only 1 hour (Jackson, Bartek 2009). UV light also has the ability to stimulate ROS, which cause oxidative stress (Synowiec et al. 2015). UV light is not the only environmental agent that causes DNA damage. For instance, factors that are produced by smoking tobacco are cancer-causing chemicals, these products trigger various cancers. The same goes for food, for example, burnt meat and contaminated food (Jackson, Bartek 2009). Another exogenous DNA damage-inducing factor is radio/chemotherapy. In contrast to the factors described above, in this case, DNA damage is deliberately induced. The most common treatment for cancer patients is radiotherapy (RT) and, therefore, many cancer patients receive this treatment. The aim of this therapy is to damage the DNA of the tumor cells, by making use of high-energy radiation. Another well-known treatment of cancer is chemotherapy, tumor cells are destroyed during the chemotherapy by stopping them from growing and ensure that the tumor cells are not multiplying. Healthy cells are also affected by radio/chemotherapy, which causes DNA damage in healthy cells (Riccio, Cingolani & Pascal 2015).

All these factors that cause DNA damage, which are mentioned above, lead to single strand breaks (SSBs) or to double strand breaks (DSBs) (O'Hagan 2014). The SSBs contains a DNA lesion which affect only one of the DNA strand while at DSBs, the lesions involves a break at both DNA strands (Nicolai et al. 2015). A SSB is less dangerous to a cell compared to a DSB. This is because during SSBs is only one DNA strand affected, the other strand can be used as a template. DSBs lesions are the most injurious forms of DNA lesions. These lesions can be developed spontaneously during endogenous genotoxins or they can arise in a response of exogenous genotoxins, for example ionizing radiations (Larsen, Stucki 2015). DSBs are more dangerous, because now both strands are affected in the cell, which can lead to the rearrangement of the genome. Other examples of DNA damage types; are alkylation, deamination, pyrimidine dimer, mismatches, intercalating agent, interstrand crosslink, deletions and insertions (Helleday, Eshtad & Nik-Zainal). It is important that DNA damage is detected and repaired as quickly as possible, for our own survival and for the faithful

transmission of DNA to our offspring. The mechanism which is responsible for detecting lesions is called the DNA damage response (DDR).

3. DNA damage response

DNA damage is detected by three members of the poly(ADP-ribose) polymerase (PARP) family, PARP-1, PARP-2 and PARP-3 depending on the type of damage. However members of the PARP family will also recognize DNA with DSBs, the earliest respondents to DSBs is Mre11-Rad50-Nbs1 (MRN) (Williams, Williams & Tainer 2007). Besides PARP, has MRN also an important role in DNA damage, including DNA repair, telomere fixing, DNA replication and checkpoint activation (Lamarche, Orazio & Weitzman 2010). Focusing on the PARP family, PARP-1 binds to various structures of DNA, such as DSBs and SSBs, while PARP-2 is less competent to SSBs (Sousa et al. 2012). If these members detect DNA damage then these members produce the catalytic production of poly(ADP-ribose) (PAR). PAR is a signaling molecule that has an important role in the activation/coordination of the cellular DDR (Riccio, Cingolani & Pascal 2015). The DDR is a complex mechanism, which includes DNA repair systems and checkpoints. These signals support DNA repair in a response to endogenous and/or exogenous genotoxins. It is a complex mechanism, because it includes a wide range of checkpoints, signal-transduction pathways, and effector systems. The effectors system has an important role in the cellular processes, such as cell cycle progression, transcription, chromatin remodeling, replication, differentiation or apoptosis (Nicolai et al. 2015). For example, cells with modest damage arrest in the cell cycle, at the moment when the repair mechanism starts, so these cells may survive. Cells with more grave DNA damage are not arrested in the cell cycle but are undergoing programmed cell death by necrosis, apoptosis or autophagy (Gasser, Raulet 2006). DDR is also triggered by telomere dysfunction. Telomeres protect the ends of the chromosomes by repetitive DNA structures (Tutton et al. 2015). Each cell division leads to a shortening of the telomeres, but when the telomeres reach a minimal telomere length, they lose the ability of chromosome protection which will be recognized as DBSs. The consequences of telomere dysfunction for human cells are entering senescence or apoptosis (Jackson, Bartek 2009). Depending on the damage level, the DDR mechanism will give a strong or weak response, so the DDR is not a ‘switch on, switch off’ pathway (Allen et al. 2015). The DDR can lead to cancer when the DDR mechanism develops significant defects. The aggressiveness of cancer, which is caused by DDR defects, depends on the amount of defect in the DDR mechanism. If there are more defects in the DDR mechanism, the cancer will be more aggressive (Karanika et al. 2015). The reason is because it has an important function in repairing the DNA damage. When this mechanism contains defects, the DNA damage remains unrepaired while the chance of developing cancer will grow. Thus, the DDR mechanism is an important mechanism in cells; one major reason is the ability of repairing DNA damage in cells.

4. Repair systems

As mentioned above, the DDR is a complex mechanism of DNA damage detection and repair systems, with each repair system devoted to a specific type of damage. ATM- and Rad3-Related (ATR) and Ataxia Telangiectasia, mutated (ATM) are key mediators of the DDR (Abraham 2001). ATR and ATM are activated by different types of DNA damage, ATR is induced by SSBs, while ATM is activated by DSBs (Purvis 2012). Although ATR and ATM are activated in different ways, their mediators have a functional overlap in the checkpoint activation. ATR and ATM work together with checkpoint mediators and kinases Chk1 and Chk2. Chk1 is activated by ATR while Chk2 is activated by ATM. In the absence of DNA damage, Chk2 is inactive and becomes activated only when ATM response to DSBs. By means of the variety of 'crosstalk's' between these kinase it is not always like the original concept of the activation as describe above (Bartek, Lukas 2003). ATM is in general the most important mediator of the G1 checkpoint, while ATR is generally regarded to be the most important mediator of the intra-S-phase and G2/M checkpoints. Even so, some studies show that ATM also plays a part in the activation of the intra-S-phase and G2/M checkpoints, depending on the type of DNA damage (Weber, Ryan 2015, Callen, Nussenzweig & Nussenzweig 2007).

There are various different repair mechanisms for DNA double strand breaks; homologous recombination (HR) and non-homologous end-joining (NHEJ) are two examples of these mechanisms. These examples are the two major repair mechanisms for DSB. A lot of products have an important role in HR, which is therefore, a complex mechanism. During NHEJ, which is less complex than HR, Ku protein has the major role. This protein ensures that an open ring structure is formed that is able to bind onto one side of the DNA strand break. One side of the ring is closed for protecting the DNA, while the other side is more open to other, so other NHEJ factors which could enter the DSB (Jackson 2002). Other repair systems for DNA repair types other than DSBs are base-excision repair (BER), nucleotide excision repair system (NER) and mismatch repair (MMR). BER exist of two general pathways, the short-patch and the long-patch. BER ensures the integrity of the genome by repairing many different types of DNA damage in different cells. The center of the BER system only consist of four proteins, which include a DNA glycosylase, an AP endonuclease, a DNA polymerase, and a DNA ligase. These proteins remove the incorrect DNA base and replace this base with a correct one (Robertson et al. 2009). Short patch has the ability of only repairing one nucleotide at the time whereas the long patch repairs more than one (Sattler et al. 2003). Another example of repair system is NER. NER requires around 25 proteins for recognizing the DNA damage, opening the DNA helix around the damage, and cutting the damaged strand on both sites of the lesion (de Boer, Hoeijmakers 2000). NER is able to recognize and repair a large variety of DNA damage (Zhang, Rohde & Wu 2009). The last example of the repair systems is MMR. MMR targets base-base mismatches and insertion/deletion loops (IDLs) that give rise to frame shifts (Schofield, Hsieh 2003). Thus DNA-damage can be repaired by different repair system as mentioned above, but these are not the only

important mechanisms in the cell. Another important mechanism is cellular senescence, which plays an important role in the prevention of cancer.

5. Cellular senescence

The proliferation of tumor cells can be permanently arrested by an important mechanism called cellular senescence. Senescence arises in the response to oncogenic stress mediators, DNA damage or by the critical minimal length of the telomeres (Kuilman et al. 2010). The growth arrest by senescence is permanent, this means the cells are irreversible blocked for growth. Senescence cells are different from other non-dividing cells, such as terminally differentiated cells and quiescent cells. The main characteristics of senescence cell are: the growth arrest is irreversible, the cells secrete β-galactosidase (SA-βgal), p16INK4a, the secretion of numerous proteases, cytokines, growth factors and other proteins which called senescence-associated secretory phenotype (SASP) (Rodier, Campisi 2011). SA-βgal is active in senescence cells, but not in quiescent fibroblasts or pre-senescence cells, therefor SA-β gal is used as a biomarker for senescence in vitro as well as in vivo.

There are three different types of senescence; replicative senescence, stress-induced senescence and oncogene-induced senescence (OIS). The replicative senescence is an essential aspect of somatic cells. Most of the tumor cells are an exception of this. Replicative senescence is connected to the DDR, but also to RB and p16INK4a. The second type of senescence is the stress-induced senescence. This type is a response to ionizing radiation and oxidative stress and DNA damage reagents, such as chemotherapeutic genotoxic drugs. These types of stress are enough to trigger the inducing of the cell growth arrest by senescence. The last type of senescence is the OIS. This type of senescence can occur independently or by telomere shortening whereby mainly inducing cell growth arrest in young cells. Tumor cells at early stages already show OIS (Kojima et al. 2013). All these three different types of senescence are connected to the DDR, because of the critical minimal length of telomeres. ATM, ATR and DNA damage kinases are activated and bring a DDR response. ATM and ATR activate two other kinases, CHK1 and CHK2 which phosphorylate p53 and CDC25. This will lead to cell growth arrest and DNA repair. If the DNA damage cannot be repaired, cells undergo senescence or apoptosis.

Cellular senescence is activated by effectors, such as a small group of cyclin-dependent kinase (CDK) inhibitors. The CDK inhibitors can be divided into two groups, the Cip/Kip family and the INK4 family. The Cip/Kip family consists of three different proteins, these proteins associate CDK4-6/D and CDK2/E-A kinases. The effect of the Cip/Kip family depends on the amount of the CDKs. If there is enough of the Cip/Kip proteins, these proteins will bind to the CDK2/E-A kinases and this will lead to cell cycle arrest.

The other group of the CDK inhibitors is the INK4 family, which consists of four members. p16INK4a is the most important member of the INK4 family (Bringold, Serrano 2000). p16INK4a upregulates the response to oncogenic stresses and senescence. When p16INK4a

is induced by oncogenic stress for example, this will activates retinoblastoma tumor suppressor protein (Rb).

Thus, a variety of stimuli can generate a senescence response, beside that the cells appear to converge into only one or both pathways to arrest the senescence growth. These two pathways are governed by tumor suppressor proteins, p53 and pRB.

5.1 P53

Another important pathway in de cellular senescence is the p53 pathway. One of the key mediator in the senescence response is p53, which is activated by DNA damage (Campisi 2005). p53 is a transcription factor, where CDK inhibitor p21 the most important target is (DiGiuseppe et al. 1995). P53 has a wide range of cellular mechanisms, such as cell cycle repair, apoptosis, DNA repair, and senescence. The E3 ubiquitin-protein ligase and the dysfunction of telomeres ensures a regulation in the p53 pathway, which stimulate a DDR response and that induce senescence. p53 pathway and the DDR ensures a defense mechanism against cancer by arrest the cells with DNA damage (Campisi, d'Adda di Fagagna 2007). The senescence of cells were delayed or abrogates by the loss of p53 (Kim et al. 2015). So although the physiological signals cannot reverse the growth arrest in the senescence cells, in some cells the loss of p53 function can lead to reversible senescence.

Although p16INK4a seems to play a more outstanding role in human cells than p53. However, if p16INK4a-RB and the p53 pathway are both activated, it induces senescence in different human cells (Kuilman et al. 2010).

5.2 Rb pathway

The CDK4/-6-Cyclin D kinase complex is the main regulator of Rb. If Rb is unphosphorylated it associates to numerous transcription factors and silence the transactivation functions. The E2F is a major factor in the Rb pathway, it activates DNA synthesis. As shown in figure 1, the p16INK4a inhibit the CDK4-6D which provides Rb in an unphosphorylated form which repress heterochromatin at loci containing E2F targets and lead to irreversible senescence growth arrest (Campisi 2005).

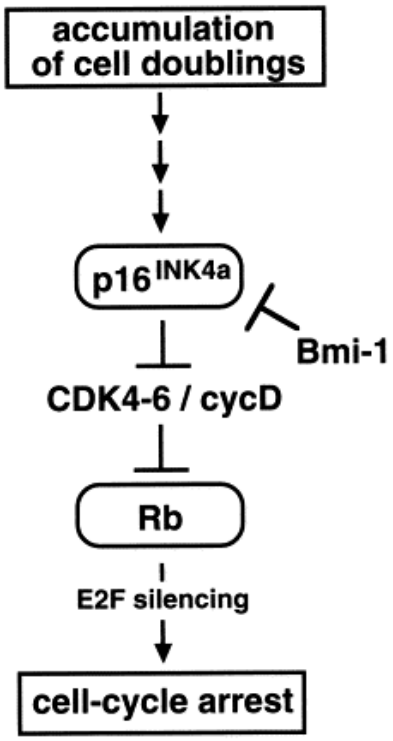

Figure 1: ***Senescence cell cycle arrest mechanism.*** *The accumulation of cell proliferation causes increased secretion of p16INK4a, which can be blocked by Bmi-1. p16INK4a has the ability to inhibit the CDK4-6D, which is a leading cause for unphosphorilation of Rb. Following, occurs the repression of heterochromatin at loci containing E2F. When the cell has reached this stage the irreversible cell cycle arrest will take place.*

5.3 Secreted factors in senescence

Intense changes are found in the transcriptomes of the senescence cells. This is because the large amount of factors, such as chemokines and cytokines are being released in this process. Various studies have disclosed the development from the senescence-associated secretory phenotype (SASP) complex in senescence cells. This complex can affect the behavior of the neighbor cells. SASP promotes endothelial cell invasion, interrupts normal mammary differentiation and stimulate cell growth in tumors. There are also SASP factors that can reinforce by the mechanism of paracrine and autocrine senescence arrest. The different functions of the SASP complex depends on the physiological circumstances (Kang et al. 2015).

Pro-inflammatory cytokines are also secreted by senescence cells, these factors trigger a range of cellular responses (Kuilman et al. 2010). Besides that, senescence cells also stimulate adjacent premalignant and malignant cells to growth and form new tumor cells. In contrast, senescence cells also secrete high levels of interleukin 6 (IL-6) and interleukin 8 (IL-8), which stimulate the immune system. The immune system than repairs the DNA damage in normal tissues but also inhibit the tumorigenesis (Rodier, Campisi 2011).

So in conclusion, there are healthy cells, which can get damaged and develop lesions by exogenous and endogenous factors. The DDR is a complex mechanism which detects and repairs these lesions. When the DDR does not repair the lesion or repairs incorrectly, than these cells accumulate their DNA damage. By secretion of different signals, cells can undergo senescence or apoptosis. In the worst case these cells with accumulated DNA damage become tumor cells. This cellular overview is shown in figure 2.

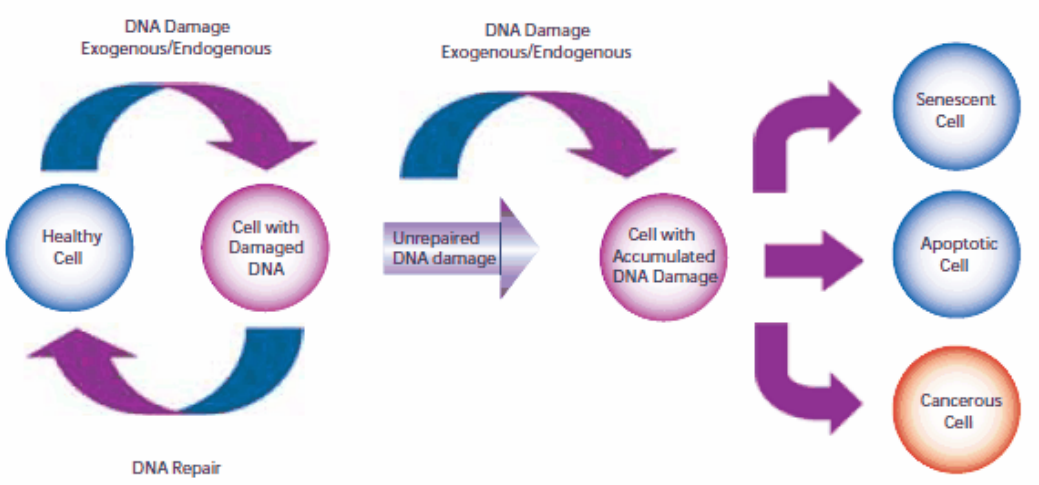

Figure 2: ***Short summary of cell mechanism.*** *Exo- and endogenous factors can lead to DNA damage in healthy cells. When DNA repair fails the cells will accumulate the damaged DNA. This process can lead the cells to become senescent, apoptotic or cancerous state* (Sigma).

6. Tumor repopulation complex

DNA damage triggers the upregulating activity of the caspase 3/7 protease, which leads to apoptosis and the production of prostaglandin E_2 (PGE_2). PGE_2 is indirectly involved, through stimulating tumor angiogenesis, in the progression and the growth of tumor cells. This will lead to an increase of the tumor population (Fan, Wang & Wang 2015). The secreting factors of dying tumor cells have the ability to trigger the neighbor tumor cells to survive and repopulate, this phenomenon is called the Phoenix Rising process. The Phoenix Rising process is compared to wound healing (Allen et al. 2015). Figure 3 shows the process of the Phoenix Rising mechanism.

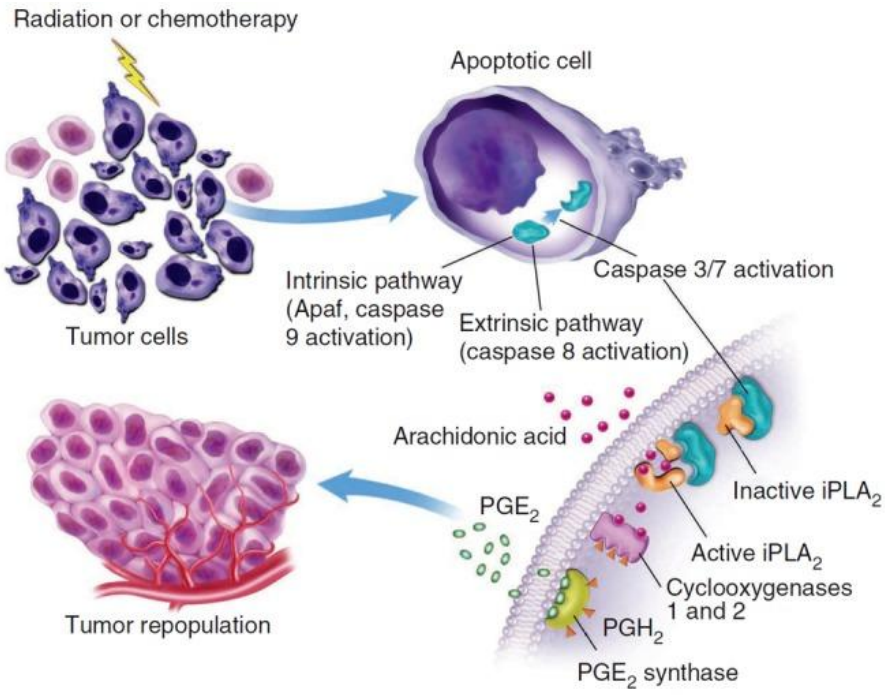

Figure 3: ***Phoenix Rising process of tumor cells.*** *When tumor cells get damaged by radiation or chemotherapy, they secrete active caspase 3/7. Caspase 3/7 interacts with $iPLA_2$ and activates it. Active $iPLA_2$ allows the intake of arachidonic acid, which is a metabolite for COX1 and 2 and is converted into PGH_2 . This molecule is converted by PGE_2 synthase into active PGE_2. Secreted PGE_2 positively stimulates the formation of new blood vessels ,angiogenesis, in the tumor. This leads to growth and progression of the tumor. Phoenix Rising results in growth and repopulation of the tumor by using the factors of its own apoptotic cells (Liu, Li & Yuan 2014).*

7. Cancer therapy

Radiation and chemotherapy are the most common therapies in the treatment of cancer. The tumor microenvironment (TME) is important for the effect of the radiotherapy. The TME is the interaction between the stroma and the tumor itself. Radiotherapy induces numerous processes in the TME, such as: fibrosis, cycling hypoxia, inflammation, revascularization and immunomodulation (Barker et al. 2015). The first use of the chemotherapy treatment in cancer started in 1940. Through several years, al lot of effort has been put into the research for optimization of the chemotherapy. In the 1963, George Canellos and Vincent DeVita started with using a combination chemotherapy, an example is the treatment for Hodgkin's Lymphoma were they used the MOPP regimen (vincristine, nitrogen mustard, prednisone and procarbazine) (Chabner, Roberts 2015). Nowadays researchers are using more often combination treatments in the therapy of cancer. Karen Weintraub published in January 6th, 2016 a paper about her research on cancer treatment with the combination of immunotherapy and epigenetic drugs. In this research they used two different types of epigenetic drugs, histone deacetylase (HDAC) inhibitors and DNA-methylation inhibitors (DNMTi) (Weintraub 2016). This combination has as result to complete the activation of tumor suppressor genes (Goffin, Eisenhauer 2002). The underlying mechanism of HDAC inhibitors is unclear, but HDAC inhibitors induce cell death in tumor cells, whilst normal cells stay unaffected (Adams, Hiebert & Eischen 2015). DNMTi activates the DDR and it ensures the prevention of a methyl groups on genes in tumor cells. If the genes in the tumor cells are silenced by methylation, it will lead to a slow cell division. The result of this is modifying the uncontrolled cell growth. Thus the DNMTi will inhibit the uncontrolled cell growth in these tumor cells (Weintraub 2016, Jin, Robertson 2013).

In conclusion, the radiotherapy is related to secreted factors and the DDR. As mentioned above, the TME is important for the effectiveness of the radiotherapy. The TME secrete different factors compare to non-tumor cells. Radiotherapy affect not only the tumor cells but also healthy cells. The DNA damage that arise, trigger the activation of the DDR. The combination treatment of Karen Weintraub is also related to the DDR, because as mentioned above, the use of DMTi activates the DDR.

8. Discussion

Cancer is according to the WHO the leading cause of death worldwide, millions of people are diagnosed with cancer and most of them eventually die from it. Cells communicate with each other by secreting signals. The knowledge of the signaling of tumor cells may help the scientific world to develop a new treatment to cure cancer. One major characteristic of cancer is uncontrolled proliferation. A known mechanism which prevents cell proliferation in tumor cells and damaged cells is called cellular senescence (Weintraub 2016). This mechanism induces irreversible growth arrest. If this mechanism is induced in tumor cells it prevents tumor cells of proliferation. Since each type of cancer need a unique treatment it is necessary to develop new cancer treatment.
Therefor was the main question in this thesis: Do factors secreted by cells after DNA damage and the development of cellular senescence potentially lead to a cancer treatment?

The microenvironment in tumors could be a good target for a treatment for tumor metastasis and tumor growth. Non-tumor cells, such as parenchymal and stromal cells in the microenvironment of the tumor can lead to progression through crosstalk with other non-tumor cells, extracellular matrix (ECM) and tumor cells. During this crosstalk a variety of secreted factors achieved from different cell types, which encourage cell growth. The most important part of non-tumor cells is that these are genetically stable compared with tumor cells. Tumor cells undergo various mutations and are thereby less targetable than non-tumor cells. Thus, successful strategy could beat cancer by targeting non-tumor cells and the factors they secrete into the microenvironment of the tumor (Lee, Pandey & Popel 2015).

Another target for cancer therapy could be inducing of the cellular senescence, since this mechanism leads to arrest of cell proliferation. If tumor cells are undergoing cellular senescence than this could be a good way to cure cancer. Cellular senescence secrete factors, such as pro-inflammatory cytokines, numerous proteases and express p16INK4a. If these secreted factors could express in tumor cells, than these cells undergo senescence. This may lead to a reduction of the tumor cells. The transgenic mice study of Maria A. Blasco and Manuel Serrano is an example of this, this study shows that mice in senescence, which express more INK4 and p53, has a better protection against cancer without any disadvantageous side effects (Serrano, Blasco 2007). Thus, if for example in tumor cells the p53 expression gets reactivate than cells have a better protection against cancer. K.G. Wiman showed that PRIMA-1 and nutlin are already being successfully tested as reactivator of p53 (Acosta, Gil 2012).

As mentioned above, the minimal length of the telomeres is also an activator for a cell to undergo senescence. 90% of tumor cells have a telomerase activity which increase the ability of developing cancer. If for some reason this telomerase activity can inhibit than the tumor cells are not able to increase. Various experiments have investigated the best ways to inhibit the telomerase function in tumor cells. So far, the best way to inhibit the telomerase activity

is to use small inhibitor enzymes (GRN163L). This technique is not without side-effects, the study of Calvin B. Harley shows that the use of these small inhibitors could lead to gross apoptosis and aneuploidies (Harley 2008).

In tumor cells the function of apoptosis does not work anymore, as result these cells still divide and the tumor gets bigger. If a drug can induce apoptosis in these cells tumor growth can be reduced. It is necessary to use a drug that is not toxic and have less impact on the normal human cells. Henry C.Lai and Narendrai P. Singh investigated the apoptosis caused by Artemisinin (used as malaria therapy) in human tumor cells. Artemisinin forms free radicals when the peroxide reacts with iron. Tumor cells have a high range of number of transferrin receptors compared to normal cells. Therefor Artemisinin is more selective to attack the tumor cells instead of normal cells because of the higher iron uptake (Singh, Lai 2004, Lowe, Lin 2000).

A major characteristic of the development of cancer is the interruption of the DDR mechanism. The DDR activate various cellular responses/activities. The more defects in the DDR mechanism, the more aggressive the cancer will be. With a potential drug for the treatment/preventing of defects in DDR, there will be a potential way for reducing cancer developing. At least 450 genes are integral to the DDR. The type of DNA repair is important to optimize the drug target in the DDR mechanism, since the DDR includes various DNA repair mechanisms. For example, when the tumor cells are sensitive for DNA-damage, this could lead to apoptosis which will lead to death of the tumor. Tumor cells can be sensitized for DNA-damage by using specific inhibitors. The use of DDR inhibitor in tumor cells could prevent the activation of the repair mechanism. These tumor cells will then undergo apoptosis, preventing the DNA repair thus accumulation damaged DNA in the tumor cells. Various compounds are under clinical trial that target the DDR mechanism. Checkpoint kinase 1 and 2 (CHK1/2) and WEE1 are protein kinases that plays a role in the checkpoint of the cell cycle and are induced under the conditions of replicative stress and DNA damage. Magnussen et al. showed a DDR-inhibitor treatment in the cure of cancer. The combination of CHK1/2 and WEE1 inhibitors showed a reduction in the tumor growth and it leads to DNA-damage followed by apoptosis (Magnussen et al. 2015). As mentioned above the DDR has various DNA repair mechanism, so there could be also various different target drugs to function as a therapeutic treatment of cancer, such as PARP-1. DNA damage is detected by the PARP family. PARP inhibitors play an important role in the cure of cancer. The first clinical test of the PARP inhibitor was Olaparib, which was successfully used in the treatment for breast cancer.

In conclusion, a complete knowledge of the development of cellular senescence and the factors secreted by cells due to DNA damage may lead to new treatments to cure cancer.

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