Cancer cell proliferation being manipulated by SSRIs via multiple pathways, resulting in cancer cell apoptosis

Determining the function of SSRIs on intracellular pathways managing cancer cell proliferation, cell cycle arrest and apoptosis

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Abstract

Background There are indications that SSRIs have a dual-purpose in cancer treatment. Firstly, they target depressions and secondly they are involved in multiple intracellular pathways involved in (cancer) cell proliferation and apoptosis.

Results It has been shown SSRIs activate multiple pathways involved in the induction of cancer cell apoptosis, including the Raf-Ras-MEK-ERK pathway, the JNK pathway and SSRIs induce excessive Ca^{2+} influx. Activation of these pathways leads to increased amounts of apoptotic factors like caspase-3, caspase-7 and caspase-9. Additionally, fluoxetine enhances the function of chemotherapy in aggressive glioma brain tumours. Ultimately, SSRIs increase levels of cell arresting compounds like p53 and p21 proteins.

Conclusion SSRIs have a dual-purpose in cancer by both targeting depression and cancer cell proliferation. SSRIs, at least fluoxetine, have potential as an enhancer to chemotherapy treatment.

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Introduction

Cancer research is under constant innovation and it is a global, unified goal to develop cancer treatments not only being effective, but also sustainable for both the survival rate of patients and the quality of life. Currently, surgical removal, radiation and chemotherapy are the common types of cancer treatments and success rates of these methods fluctuate on a scale from very effective to no effect. The high uncertainty accompanying these therapies, and also considering the side effects, makes finding new cancer therapy approaches the everlasting goal.

Because advanced technology provides scientists with a constant flow of new information, it seems that more advanced imaging techniques and research tools allow researchers to further zoom into the behaviour of the cell. As is known, cells undergo influences from a great number of pathways involved in cell growth and survival. Following from this is the knowledge that cell division, in both healthy and cancer cells, is regulated by a multiple of pathways. It makes sense that manipulating these pathways will trigger changes in cell behaviour and could influence the processes of cell division (of cancerous cells) and the proliferation and growth of tumours. It is the goal of this essay to understand the function of SSRIs in these processes.

Why would SSRIs influence cell proliferation and tumour growth?

SSRIs are compounds prescribed to patients suffering from depression and/or anxiety disorders. They are approved, well known, and commonly acknowledged compounds. The reason SSRIs could be related to cancer is because there are indications describing the process of SSRIs manipulating intracellular pathways involved into the processes of cell division and cell proliferation, for example inhibiting growth and inducing an apoptotic effect in colorectal carcinomas (Xu et al., 2006; Gil-Ad et al., 2008), lung cancer (Toh et al., 2007), ovarian cancer cells (Lee et al., 2010), and in lymphoma cells (Frick et al., 2011).

It is the objective of this essay to dig into the function of SSRIs and describe the mechanisms behind SSRIs restricting cell proliferation. Because of the great complexity of pathways and the indication that SSRIs target multiple pathways, several of them will be analysed, these include the ERK pathway, the influx of Ca^{2+} and the JNK pathway. These different processes are involved into the survival, apoptosis and proliferation of both healthy and cancer cells. SSRIs interact with these pathways and therefore play a role in these processes.

Current SSRI intake and dual-purpose

SSRIs and cancer are not strangers, because cancer is a reason to trigger depression (Brown et al., 2010). For that reason cancer patients are already prescribed SSRIs (for example fluoxetine). SSRIs are already administered to cancer patients and fortunately the prescription of those does not negatively influence the classic cancer treatments (Ostuzzi et al., 2018). On the contrary, multiple times it has been indicated how SSRIs facilitate cancer treatments and are in fact beneficial for the cancer treatment procedure.

It is important to note that SSRIs are already known for their function in depression (by blocking the reuptake of 5-HT), and this effect is achieved in cancer patients suffering from depressions, increasing their quality of life and life perspectives. However, that is not the effect investigated into in this essay. Nevertheless, this effect should not be forgotten because it has already been demonstrated how cancer patients suffering from a depression benefit from taking SSRIs.

Shortly, SSRIs seem to have a dual-purpose in cancer. Firstly targeting depression and therefore increasing quality of life and secondly manipulating cellular pathways involving tumour proliferation. The objective of this essay is to examine the second function.

1. Introduction to cancer

The severity of cancer is common knowledge. Despite better healthcare, advanced technology and increased knowledge, cancer persists to be one of the major causes of death (Siegel et al., 2017). Mainly because of the increasing prevalence directly caused by the ageing society and aggressiveness of the tumours. Research learns that cancer and genomics are heavily connected. It leads to the conclusion that cancer development and proliferation has its roots in mutations in the DNA (MacConaill et al., 2010).

Malfunction in cell cycle arrest

The role of gene mutations seem to play an important role in the onset of cancer. This expresses itself in cancer cells being able to overcome the processes to eliminate DNA damage. Examples are apoptosis and 'cell cycle arrest', the phase of the cell cycle that screens for DNA damage and 'arrest' the cell cycle, preventing the cell to grow and exit the cell cycle process. This process is induced by the p53 gene and it is clear that mutations in the p53 gene can cause cells with DNA damage to leave the cell cycle and thereupon divide onwards without restriction, and from that point on become autonomous functioning cells with the characteristics of tumour cells (Hollstein et al., 1991; Brown et al., 2009).

Three tumour development phases: initiation, promotion and progression

This p53 gene is a so called tumour suppressor gene (Hollstein et al., 1991). The definition of these genes is that they suppress proliferation. Together with its counterplayer, the oncogenes, that with activation can cause tumours, they are crucial for the body to prevent tumour growth. In the example of p53, it screens DNA on damage and if mutations are present, the p53 protein will be activated. In the resting state p53 is bound to the mdm2 protein. In the case of mutations the p53-mdm2 complex will separate and the p53 will become active and will arrest the cell cycle. At that moment there are multiple options. Firstly, p53 can repair the DNA damage and restart the cell cycle. Secondly, if the damage is severe, it can cause apoptosis and kill the cell, making sure the DNA damage will not be inherited by future cells (Hollstein et al., 1991; Brown et al., 2009; Kruse et al., 2009).

The malfunction of this p53 gene is necessary for tumour cells to proliferate. Once this is achieved and cells with mutated DNA will leave the cell cycle, the first phase of tumour development has passed: the initiation phase (Pitot, 1993; Fearon, Vogelstein, 1990).

Secondly, there is the promotion phase. In this phase the precancerous cells have the ability to become tumours by different promotors. These promotors are factors that will make sure the cells can divide without problems. In those cases the cells will be given the necessary nutrients and they need to grow, for example growing factors (Pitot, 1993; Fearon, Vogelstein, 1990).

Thirdly, the cell will enter the phase of progression. After the provision of promotors the precancerous cells have the needs to grow and divide without restrictions, and so they will: resulting in growth of tumours and autonomous division of cells without inhibition. In this phase other processes will occur, for example angiogenesis, the generation of an infrastructure of blood vessels around the tumour. This process is important in the progression of a tumour because those cells divide in a high speed and therefore need high amounts of nutrients and oxygen. Furthermore, these blood vessels allow the tumour to distribute cells to the rest of the body, causing metastasis (Fearon, Vogelstein, 1990; Michor et al., 2004).

Approaches to tackle cancer

Because the development of cancer is complex and much is needed to develop tumours, this disease can be approached in different ways. This is done by many disciplines within the field of science. One approach is the inhibiting of the process of angiogenesis. It is known that several proteins induce this process: among others vascular endothelial growth factor (VEGF), tumour necrosis factor TNF-α and other growth factors (Nishida et al., 2006). The body will produce VEGF in cases of hypoxia, and tumours are prone to hypoxia because of the high speed of division and so it demands high amounts of oxygen. The concept is to block this VEGF and prevent it from functioning and therefore inhibit the development of a blood vessel infrastructure around the tumour. The idea seemed hopeful, however the results are disappointing. Anti-VEGF drugs have low influence on the survival rate and limited success (Ramjiawan et al., 2017). Other research from Vasudev et al. confirms that VEGF levels are increased in tumours, but despite that observation, anti-VEGF drugs like bevacizumab and aflibercept do not seem to be able to diminish VEGF signalling in tumours, therefore giving these drugs only limited function and effect (Vasudev et al., 2014).

More traditional approaches like chemotherapy, radiation and surgical removal of the tumour are still common treatments, but seem to be, along with therapies like the VEGF inhibiting, approaches that lack the genomic point of view.

To deal with tumours from its roots, a more genetic approach is needed. In this case treatments should be developed that interfere into either of the phases of initiation, promotion and progression. Much research is done in these fields and for as it seems now, the genomic component of tumours is complex, it also offers multiple entrances to approach this problem (Fearon, Vogelstein, 1990; Michor et al., 2004).

How do cancer cells work and behave?

Understanding how cancer cells work seems the goal of research. Cancer stem cells (CSCs) are prominent in this research, because of their role in cancer: they are the foundation of a line of cells and are the safe haven to build (cancer) tissue around. They are crucial for the longevity of tissues and therefore are crucial for survival of tumours (Beck et al., 2013; Kreso et al., 2014).

Targeting CSCs seems the way to eliminate the problem at its roots. However, the study of CSCs is relatively new and therefore the behaviour of these types of cells is not completely understood. On the other hand, there has been done extensive research on regular cancer cells and its intracellular pathways involving cell division.

In this essay there will be mainly be spoken about regular cancer cells, however it is important to note the importance of CSCs and their potential to be 'immortal' (Soltysova et al., 2005). It has also been found that chemotherapy treatment could kill regular cancer cells, but CSCs seem to be quite resistant to those kinds of treatments (Soltysova et al., 2005). Widely it is agreed that genetics are the concept of study in cancer, and especially in CSCs. Discovering (novel) intracellular pathways involving the process of cell division could be the way to not only target and kill regular cancer cells but also CSCs. Even though CSCs are resistant to chemotherapy and other drugs, their cell cycle might still be vulnerable to external factors.

2. Introduction to selective serotonin reuptake inhibitors (SSRIs)

Selective serotonin reuptake inhibitors are compounds that inhibit the reuptake of serotonin in the synaptic cleft of presynaptic neurons by blocking the SERT transporter (Davis et al., 2016). This SERT transporter is the doorway that lets through serotonin to enter the neuron again and let itself being broken down or being enclosed by a vesicle to stand-by for a new transport or to store as reserves. Supplying SSRIs makes sure the SERT transporter is blocked and serotonin will not re-enter the cell but instead remain in the synaptic cleft to increase the levels of serotonin that will be absorbed by the postsynaptic cell, resulting in an enhanced signal transmission.

Because of the function of increasing serotonin, it is prescribed mainly to patients that suffer from a spectrum of disorders in which a serotonin deficit causes symptoms. The list of these disorders contains among others: depression, obsessive-compulsive disorder (OCD), anxiety disorders and eating disorders (Vetulani, Nalepa, 2000).

Characteristics of SSRIs

SSRIs are a collection of compounds that function in the same way. Its list is long and contains around 20 different drugs (of which a selected amount are approved and distributed as drugs), with most known fluoxetine, citalopram, paroxetine, sertraline and fluvoxamine (Ferguson, 2001).

Depression and prescribing of SSRIs to cancer patients

SSRIs are prescribed to cancer patients because they are prone to suffer from depression (Brown et al., 2010). The question rises what effect this depression has on the survival rate of cancer patients. This effect was researched in lung cancer patients. It was found that depression lead to a decreased survival rate (Sullivan et al., 2016). Considering this effect, it makes sense to prescribe SSRIs to cancer patients to tackle the depression. In hospitals this is done often (Ostuzzi et al., 2018).

Another study confirms this and states that SSRIs (in this case citalopram) doesn't negatively interfere with regular cancer treatments of breast cancer patients (Lash et al., 2010). In 2009 the same result was found for breast cancer and it was even found that paroxetine (SSRI) had a reduction in risk of getting breast cancer (Wernli et al., 2009).

It is the next goal to investigate into the exact effect of SSRIs and how they interact with cancer cells.

3. Introduction to pathways that link SSRIs to cancer

What we know about SSRIs until now could help us digging into its other functions. To first examine those other functions, we should consider the ability they have to bind not only to SERT but to other receptors, which are not necessarily only located on neurons, but also on regular cells, including cancer cells.

For their structure they target multiple kinds of receptors. Each of them leading to the activation of some sort of pathway. That learns us that SSRIs could induce other effects than just the ones related to the function of accumulating serotonin in neurons.

Pathways activated/inhibited by SSRIs

In this essay, multiple pathways will be analysed because there is proof that SSRIs interact with them (summarized in figure 1). For every pathway the proof of connection is stated, per receptor type. The examined pathways are:

1. Ras-Raf-MEK-ERK pathway
    a. 5-HT1A receptors activate this pathway (Della et al., 1999; Cowen et al., 1996; Chang et al., 2009).
    b. 5-HT2A receptors activate this pathway (Johnson-Farley et al., 2005; Chang et al., 2009).
    c. 5-HT7 receptors activate this pathway (Johnson-Farley et al., 2005).
2. AMPA receptor activated pathway
    a. Fluoxetine activates AMPA receptor pathways (Liu et al., 2015).
3. JNK pathway
    a. Fluoxetine activates the JNK pathway (Mun et al., 2013).

Why are these pathways important to cancer treatment?

The previous mentioned pathways play a role in either cell proliferation, cell apoptosis or cell arrest in the cell cycle, or more than one of these factors. These functions are:

1. Ras-Raf-MEK-ERK pathway
    a. This pathway plays a role in cell arrest and apoptosis (Cobb et al., 1999; Kolch et al., 2000; Asthagiri et al., 2001; Orton et al., 2005).
2. AMPA receptor activated pathway
    a. This pathway plays a role in apoptosis (Trump et al., 1995; Pinton et al., 2008).
3. JNK pathway
    a. This pathway plays a role in apoptosis (Johnson et al., 2002).

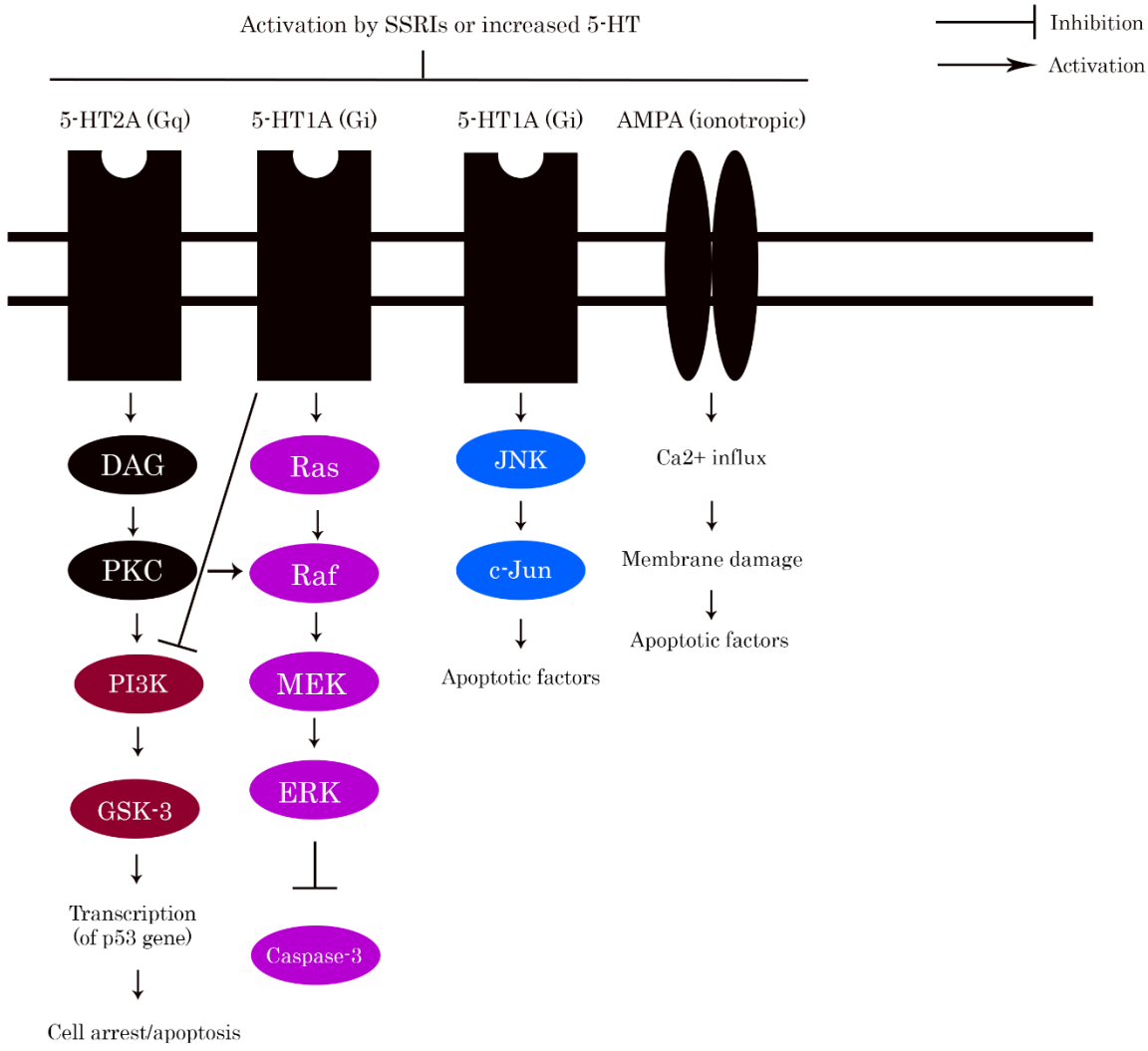


Figure 1. This image combines the information on the intracellular pathways. It is shown which proteins are part of the pathways and what their effect is. The apoptotic factors are a collection of proteins that induce apoptosis; in the rest of the essay these will be reviewed. Among others, these contain cytochrome c, caspase-9, caspase-3 and PARP (poly (ADP-ribose) polymerase), (Kalaany, Sabatini, 2009; Watcharasit et al., 2002; Cobb et al., 1999; Kolch et al., 2000; Asthagiri et al., 2001; Orton et al., 2005; Trump et al., 1995; Pinton et al., 2008; Johnson et al., 2002; Johnson-Farley et al., 2005; Li et al., 2004; Mun et al., 2013; Della et al., 1999; Cowen et al., 1996; Chang et al., 2009).

4. Introduction to Ras-Raf-MEK-ERK

ERK is a protein that is part of the Ras-Raf-MEK-ERK pathway, a chain of proteins that are heavily involved in the processes of apoptosis, gene transcription, cell division and cell arrest in the cell cycle. This pathway is activated by SSRIs, being part of different kinds of 5-HT receptors and is involved in different kinds of cancer research (Cobb et al., 1999; Kolch et al., 2000; Asthagiri et al., 2001; Orton et al., 2005). It has been shown that 5-HT1A receptors activate the Ras-Raf-MEK-ERK pathway (Della et al., 1999). Johnson-Farley also shows that 5-HT2A and 5-HT7A receptors activate these pathways (Johnson-Farley et al., 2005).

Function of ERK

The activation of the pathway starts with Ras, which will activate Raf, which will activate MEK (shown in figure 2). The MEK protein is the precursor that activates ERK and that activation will eventually lead to multiple processes. Because of the complexity of the interactions ERK has with other proteins and processes, it is heavily researched how ERK is involved in cell arrest and apoptosis (Cobb et al., 1999; Kolch et al., 2000; Asthagiri et al., 2001; Orton et al., 2005).

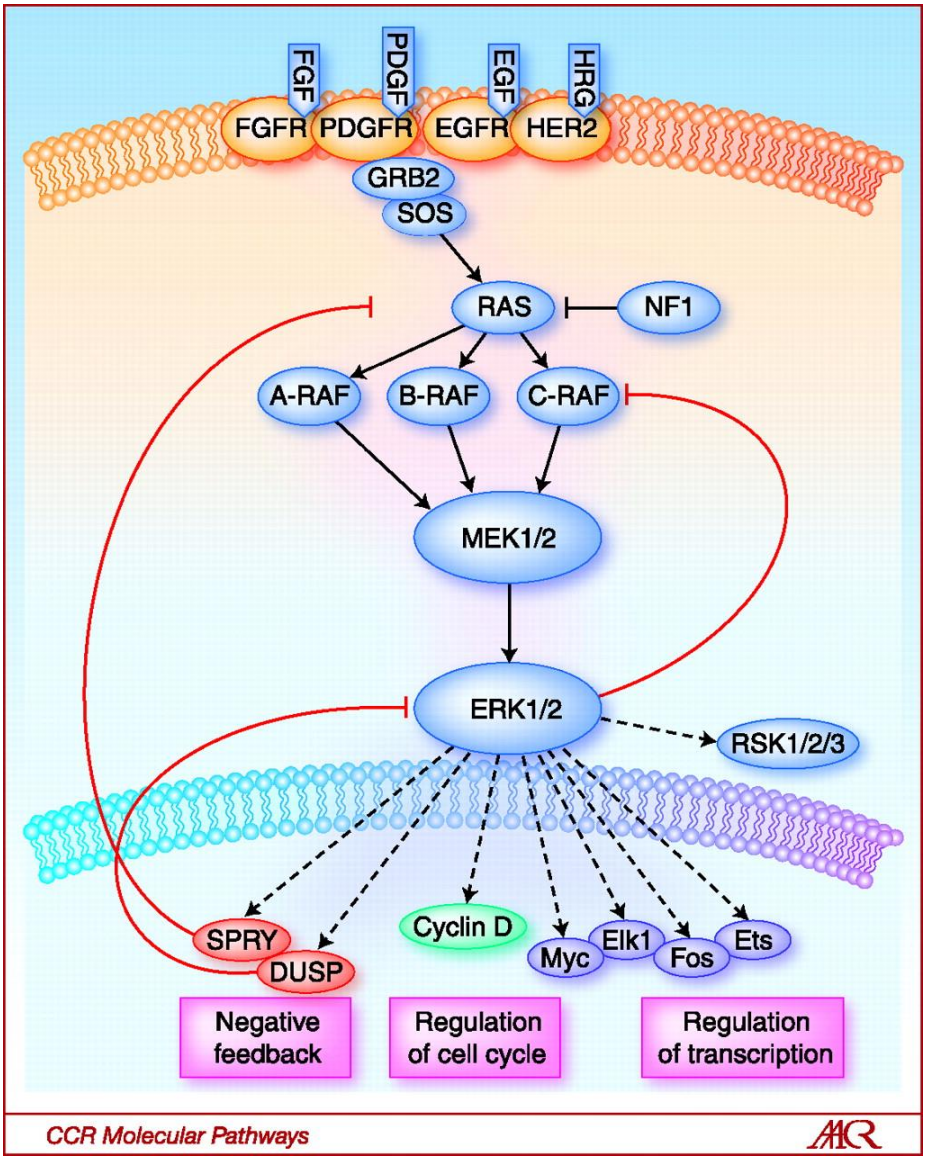
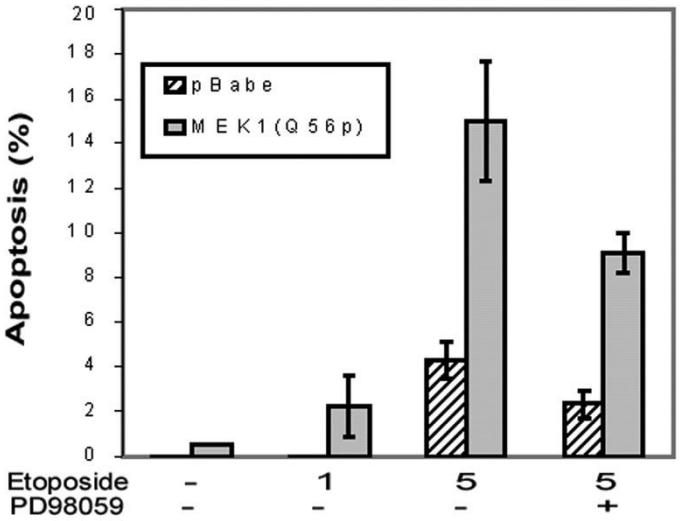


Figure 2. The RAS-RAF-MEK-ERK signaling pathway (Pratilas, Solit, 2010).

Tang et al. performed a thorough research on ERK and its relation to p53 and apoptosis. They found that ERK pathway activation induces apoptosis and ERK levels were increased in cancer, independently of p53 activity. This means the ERK pathway is an autonomous functioning pathway that either activates or inhibits. (Tang et al., 2002).

They performed multiple experiments to invest into the behaviour of cells at increased levels of ERK. It seems that ERK induces apoptosis, as seen in figure 3.



In figure 3 is visible how Tang et al. performed the experiment. They implanted the MEK1 gene into viruses (MEK1 is the precursor of ERK) and injected them into cell cultures. The viruses will express the MEK1 in the cells and the results of that expression are visualized. They measured apoptosis of the cells and compared those to viruses with a control protein (pBabe). They found significant increased values of apoptosis of cells expressing MEK1 and ERK, concluding that increased levels of ERK induces apoptosis in cell cultures.

Figure 3. Apoptosis in cell cultures exposed to MEK1/ERK and control (Tang et al., 2002).

The results from this experiment show that increased levels of ERK trigger apoptosis. It is also found that cancer cells have higher levels of ERK, which confirms the thoughts that DNA damage in wildtype cells increases ERK levels (Tang et al., 2002). In the context of SSRIs, this pathway could be used as an approach to manually increase the levels of ERK by admitting SSRIs and inducing apoptosis of cancer cells. These functions are mainly based on theoretical knowledge gathered by various studies like the one from Tang et al. Fortunately, it is a concept that is still in the picture and is researched on different occasions. Previous research of Stepulak et al. shows that there is a relationship between SSRI admission and cancer cell apoptosis. They found that in vitro use of fluoxetine inhibits proliferation of cancer cells by activation of the ERK pathway (Stepulak et al., 2008). They performed this research in different kind of cancer tissue and found for all tissues the same results. The list of cancer types investigated consists of: lung cancer, colon cancer, neuroblastoma, breast cancer and medulloblastoma. So, it seems that fluoxetine has an effect on the proliferation of cancer cells, that, so they found, already onsets within 24 hours of distributing the fluoxetine.

Moreover, they also found that fluoxetine increases levels of p21 and p53, and simultaneously decreases the activity of cyclin A and cyclin D1. Cyclin A and cyclin D1 are important proteins involved in the cell cycle. They promote cell division by letting cells pass to a next phase, inhibiting them would arrest cancer cells in the current phase and prevent them of dividing. Other research confirms this, the research group of He et al. found that in breast cancer cyclin D1 and other cyclins are overly expressed (He et al., 2013). These results suggest that inhibiting these cyclins is beneficial for cancer treatment and fluoxetine has a clear function in that inhibition. Multiple studies confirmed the overexpressing of cyclins being involved in cancer, among others in colorectal cancer (Yang et al., 2014) and prostate carcinoma (Pereira et al., 2014).

This increase of p53 induced by fluoxetine is visualized by another study, performed to measure the intracellular activity of fluoxetine. Lymphoma cells were exposed to fluoxetine, in vivo, and the p53 levels were measured, shown in figure 4 (Frick et al., 2011).

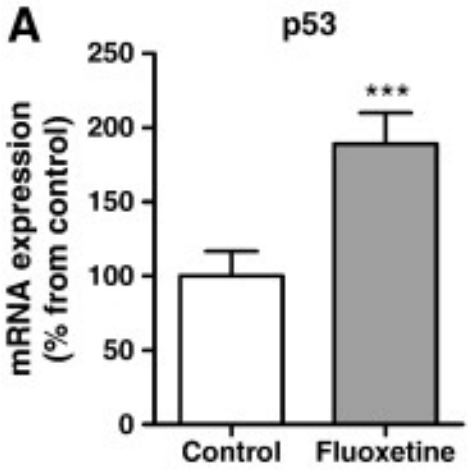


Figure 4. p53 activity is visualized by measuring mRNA expression. It is shown fluoxetine significantly increases the p53 activity, which confirms the previous mentioned studies. (Frick et al., 2011).

Figure 4. p53 activity in lymphoma in control situations and exposed to fluoxetine (Frick et al., 2011).

In the same study also activity of cyclins is measured. These cyclins are important in the cell cycle. It has been found that Cyclin D3 is significantly reduced by fluoxetine, however Cyclin D1 and Cyclin D2 activity were not affected. Low cyclin levels lead to cell arrest (Frick et al., 2011).

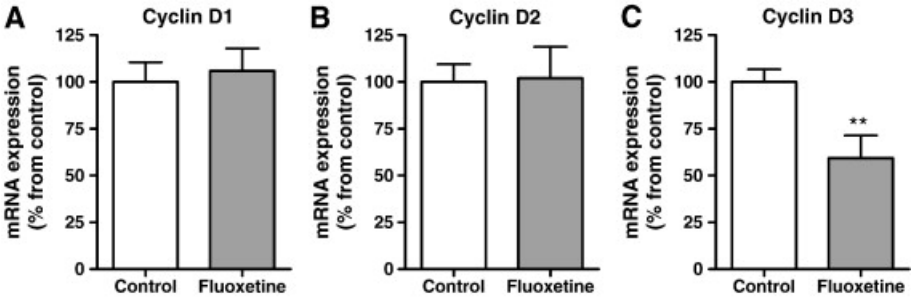


Figure 5. Activity of cyclins is measured. It is shown Cyclin D1 and Cyclin D2 are not affected by fluoxetine, however, Cyclin D3 is, leading to cell arrest (Frick et al., 2011).

Figure 5. Activity of Cyclin D1, Cyclin D2 and Cyclin D3 by fluoxetine (Frick et al., 2011).

Conclusion on ERK

SSRIs increase levels of ERK trough the Ras-Raf-MEK-ERK pathway (by serotonergic receptor targeting). ERK induces apoptosis in (cancer) cells. SSRIs will as well increase levels of p21 and p53 and decrease cyclin levels, all leading to cell arrest of damaged cell and making them being stuck in the cell cycle. This is confirmed by Frick et al., showing Cyclin D3 levels are reduced and p53 levels are increased.

5. Introduction to mitochondrial Ca^{2+} overexpression induced apoptosis

From the previous knowledge it is clear SSRIs target SERT. However, they are not limited to only those receptor types. There is proof that SSRIs also target AMPA receptors (Liu et al., 2015). These are ionotropic glutamate receptors present in the central nervous system (CNS). In the context of cancer it is important to firstly determine why binding to AMPA receptors could be beneficial and secondly prove this binding really occurs. The first question is answered by multiple papers. For example by De Groot et al. They show that AMPA receptors are present in gliomas, tumours of glial cells (De Groot et al., 2011). The second question is whether this presence is beneficial for cancer. It is common knowledge that AMPA receptor activation induces an influx of Ca^{2+} ions, which is yet again confirmed by Gruszczynska-Biegala in a recent study (Gruszczynska-Biegala et al., 2016). What effect has this increased Ca^{2+} influx on cancer proliferation? It seems that increased Ca^{2+} triggers apoptosis. This has already been shown in 1995 by Trump et al. (Trump et al., 1995). And later on this was again confirmed by Pinton et al. (Pinton et al., 2008).

An increased influx of Ca^{2+} is desired, because it triggers apoptosis and therefore it could kill cancer cells. Liu et al. delved into this subject and firstly confirmed that it is true that SSRIs increase Ca^{2+} influx, this is shown in figure 6.

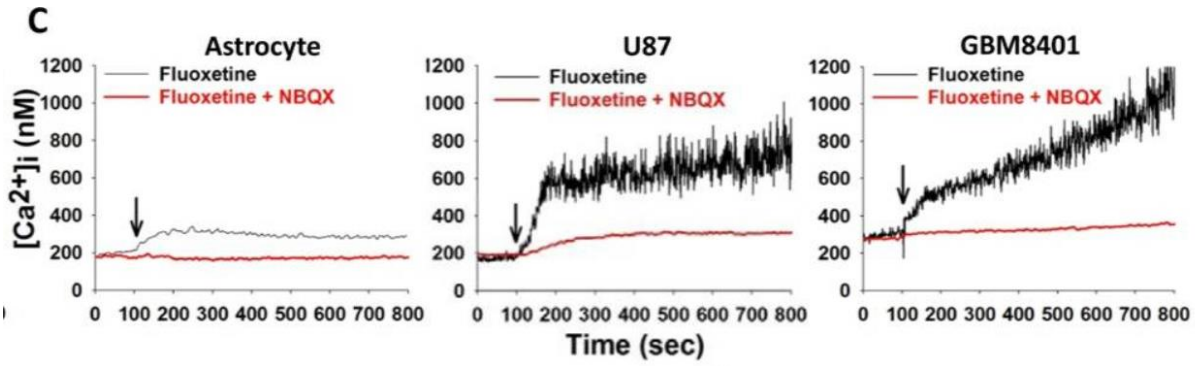


Figure 6. Confirming the influx of Ca^{2+} in brain cells in the CNS. They used NBQX as a control, which is an AMPA receptor antagonist, blocking the receptor. It is shown that with the use of NBQX there is less Ca^{2+} influx, which leads to the conclusion that in this case fluoxetine increases the Ca^{2+} influx in human astrocytes and U87 and GBM8401, which are cell lines extracted from human glioma tissue (Liu et al., 2015).

Why does increased Ca^{2+} trigger apoptosis?

Pinton et al. showed that increased Ca^{2+} influx triggers apoptosis. In their research they described the mechanism behind this. In summary, the increased levels of Ca^{2+} cause damage to the mitochondrial membrane, which leads to the secretion of apoptotic factors. The overload of Ca^{2+} influx causes organelles in the cell to swell and the mitochondrial membrane to lose its membrane potential, all leading to fragmentation of the mitochondria and eventually apoptosis (Pinton et al., 2010).

Liu et al. tried to once again confirm this effect and found the results showed in figure 7.

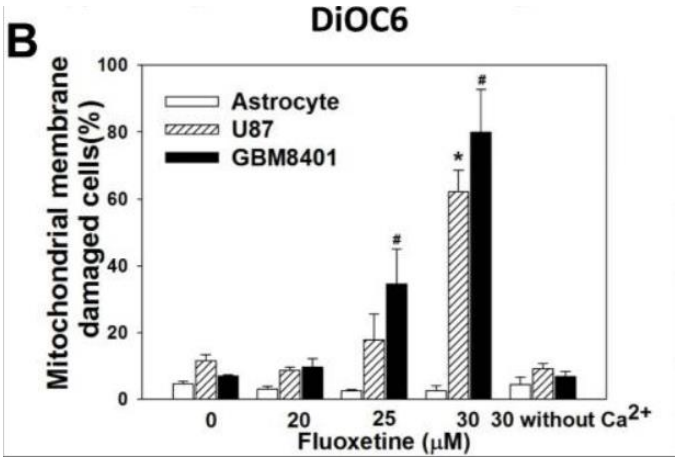


Figure 7 shows the results from an experiment on the effects of mitochondrial membrane damage caused by fluoxetine. It is visualized that in the three cell types: astrocyte (control) and U87 and GMB8401, both glioma cells, the mitochondrial membrane damage is increased in both glioma cells, but not in the healthy astrocytes. This figure shows that the previous statements of Ca^{2+} influx causing damage is true. It is beneficial that this damage only occurs in tumour tissue and not in healthy tissue.

Figure 7. Mitochondrial membrane damage caused by fluoxetine (Liu et al., 2015).

Figure 7 shows that damage occurs. How does that lead to the secretion of apoptotic factors? It seems that this mitochondrial membrane damage activates the intrinsic apoptotic route, which leads to the release of apoptotic factors, including the following factors: cytochrome c, caspase-9, caspase-3 and PARP (poly (ADP-ribose) polymerase), (Pinton et al., 2008).

Other research of Levkovitz et al. suggested the release of these caspase-9 and caspase-3 by fluoxetine and paroxetine. They stated that those factors induce apoptosis (Levkovitz et al., 2005) and those statements are confirmed by Liu et al.

It is common knowledge caspases induce apoptosis. Nevertheless, this has yet again been confirmed by Shalini et al. They performed a broad review article about caspases and their apoptotic function. As already mentioned it is confirmed that caspase-9 and caspase-3 will induce apoptosis (Shalini et al., 2015) and like many other research groups Shalini et al. tried to connect both those factors to cancer and other diseases. The paper of Liu et al. seems to connect well to the research of Shalini et al., confirming each other that such factors cause cell death and have an tremendous function in several diseases, and in this case in cancer.

Performing fluoxetine treatment on gliomas

The final phase of the research of Liu et al. is to actually admit fluoxetine to glioma cancer cell lines. The results from those admissions are shown in figure 8.

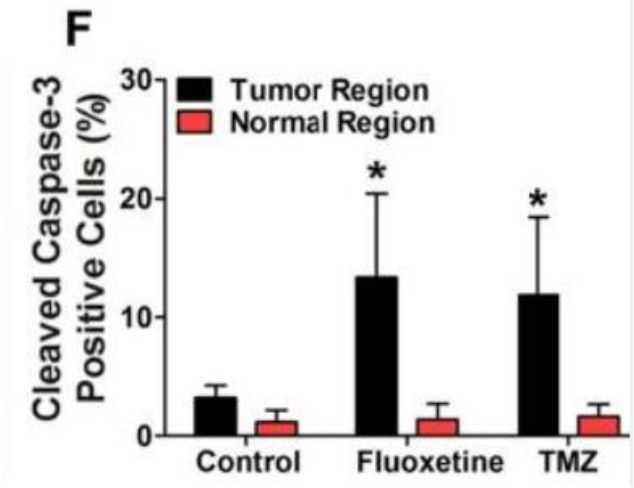


Figure 8 shows the effects of fluoxetine on tumour (glioma) cells compared to normal brain cells (astrocytes). It is seen that in the regions with tumours caspase-3 levels are heavily increased (significantly) compared to regions without tumours. As a comparison to the effect of fluoxetine they used firstly a control group, in which there was no effect of caspase-3 visible, and secondly they used TMZ (temozolomide, an approved common used chemotherapy compound used in brain tumours). Fluoxetine increases at least as many caspase-3 activity as TMZ (Liu et al., 2015).

Figure 8. Caspase-3 levels induced by control, fluoxetine and TMZ compared in tumour and non-tumour regions (Liu et al., 2015).

Tumours exposed to fluoxetine were monitored and their proliferation was imaged with the use of PET imaging. The results of the monitoring are shown in figure 9.

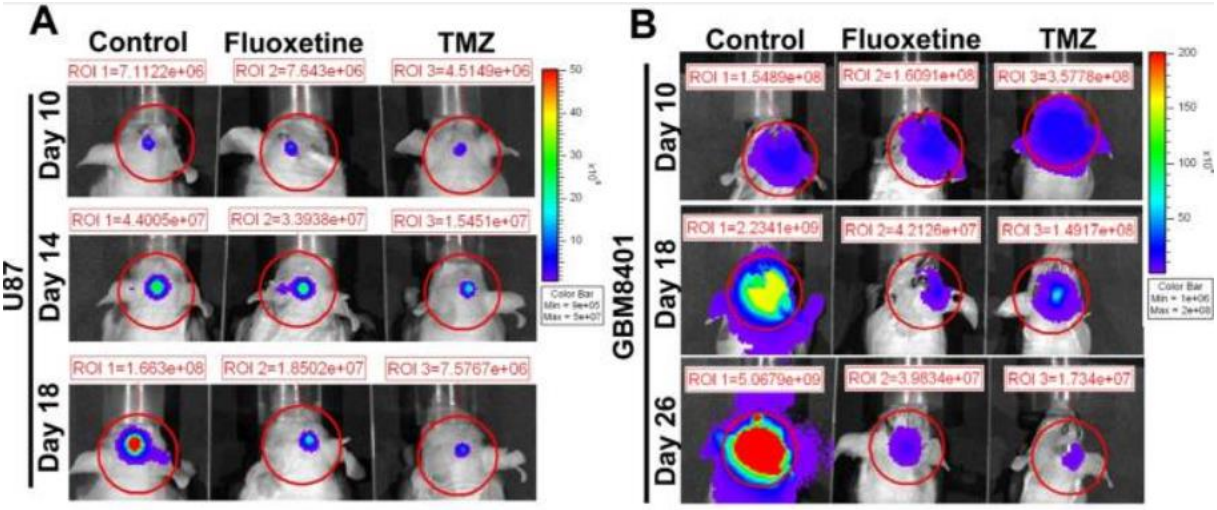


Figure 9. This image shows how in different time scales, tumours treated with the 3 different methods grow or shrink. It shows that both fluoxetine and TMZ cause a shrinkage of the tumours whereas control treatment causes proliferation of the tumour, especially in GMB8401 cell lines. Currently, both fluoxetine and TMZ are effective in gliomas (Liu et al., 2015).

Combining TMZ with fluoxetine

TMZ is the state of the art (chemotherapy) treatment in gliomas, because it is effective and it has potential to enhance its function by combining it with other drugs. TMZ is despite being effective not completely ideal, because of its side effects, like nausea and the fact it being fetotoxic and in general being toxic to the body (Neyns et al., 2010). Currently, cancer research on aggressive glioma tumours is focused on TMZ to make it more effective and less toxic to the body. Multiple attempts of combining it with other compounds were successful, for example chloroquine (normally

functional in malaria), which seems to increase sensitiveness of tumour cells to TMZ (Hori et al., 2015).

From previous results is clear fluoxetine achieves the same kind of result as TMZ and therefore it has been tried to combine both compounds and investigate into distribution of such a fusion into C6 glioma cancer cells. In this case of fluoxetine. It is interesting to note that the other effect of fluoxetine, that is the antidepressant effect, improves the status of cancer patients in a way other than the investigated apoptotic function: that is by inhibiting the depression and therefore improving the mental state and quality of life of the patients. This is an effect that should not be neglected and therefore fluoxetine is to be considered the treatment of desire, so as a matter of fact fluoxetine is already prescribed to cancer patients, not considering yet the effects it could have on inducing apoptosis (Park et al., 2015; Lauer et al., 2015).

To go back to the subject of fluoxetine combining with TMZ: this has been done by Ma et al. and the results of that trial are projected in figure 10 (Ma et al., 2016).

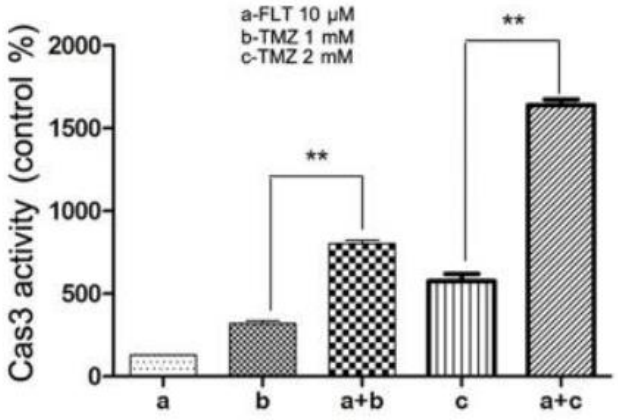


Figure 10 shows the results from administrating fluoxetine (FLT), TMZ and a fusion of fluoxetine and TMZ. The chart shows how combining both compounds significantly increases the caspase-3 activity and it is also shown how increasing the concentration of TMZ increases this effect even more. From these results follows the conclusion that combining fluoxetine and TMZ increases caspase-3 activity which leads to increased apoptosis. This combination treatment should be investigated further into to optimize Cas3 activity (Ma et al., 2016).

Figure 10. Caspase-3 activity in C6 glioma cancer cells (Ma et al., 2016)

Conclusion on fluoxetine in brain tumours

As the results from figure 8 demonstrate, fluoxetine is effective in inhibiting proliferation of gliomas in vivo in rats (Liu et al., 2015). The reason this is particularly beneficial is that especially in brain tumours drugs have trouble passing the blood-brain barrier, BBB (Preusser et al., 2011). Fluoxetine has no trouble passing the BBB and therefore is suitable in these kinds of treatments. In the case of gliomas, metastasis is a risk because of the high aggressiveness of gliomas. Metastases of these brain tumours are found in different regions of the body, for example in the lungs, breasts, colon and renal area (Kamar et al., 2010). Managing and stopping gliomas from growing could affect the process of metastasizing, and perhaps preventing it. Future research on this concept should determine the exact function of fluoxetine on managing gliomas.

Both figure 7 and figure 9 show how fluoxetine increases the apoptosis of glioma cancer cells, and combining it with TMZ gives an even more enhanced apoptosis. Considering fluoxetine is already supplied to cancer patients suffering from depression, this is something to investigate further into. Future research should compare cancer patients taking SSRIs with cancer patients not taking SSRIs, those results should give more insight in this mechanism in human patients and should theoretically inhibit the proliferation of cancer cells (in this case aggressive glioma cancer cells).

6. Introduction to JNK pathway

Another pathway coming into the picture on cancer is the JNK pathway (c-Jun N-terminal kinases, pathway). This pathway involves the activity of JNK and c-Jun proteins, the latter, c-Jun, induces apoptosis (Johnson et al., 2002). Despite that this effect already has been found in 2002, question marks remain. For example, how does JNK/c-Jun relate to cancer and what are the exact mechanisms of c-Jun inducing apoptosis?

The group Chang et al. investigated into the function of JNK/c-Jun activation on cell proliferation, apoptosis and cell viability. They performed a study in which an JNK/c-Jun agonist protoapigenone was distributed to tumour cell cultures and its effects were measured, projected in figure 11 (Chang et al., 2008).

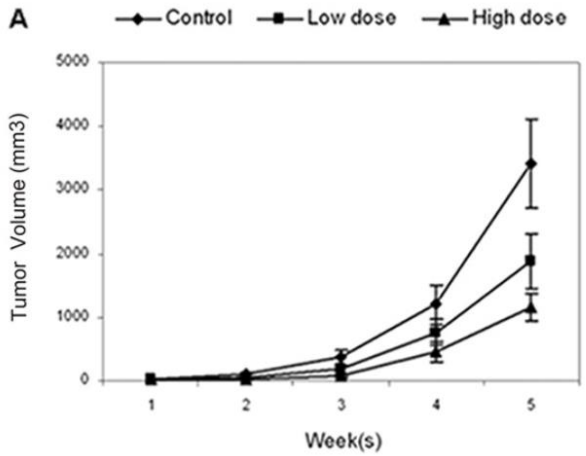


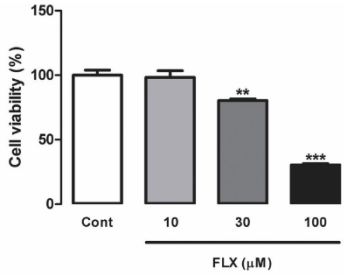
Figure 11 shows the results of exposing human prostate cancer cells to protoapigenone (the JNK/c-Jun agonist). The chart shows how protoapigenone slows down the growing of the tumour, and it simultaneously shows how higher doses of protoapigenone even further decreased the growing of the tumour. These findings lead to the statement that activation of the JNK/c-Jun pathway induces apoptosis and inhibiting of the proliferation of (human prostate) cancer cells. (Chang et al., 2008).

Figure 11. This figure shows the growing curves of tumours exposing to low dose, high dose and no doses of JNK/c-Jun agonist protoapigenone (Chang et al., 2008).

How is the JNK pathway connected to SSRIs?

Until now, this has no connection to SSRIs. However, by now it is clear that SSRIs work in many different ways and there have been indications that SSRIs do activate this JNK-c-Jun pathway. To confirm this, Mun et al. performed a study in which they screen cancer cells exposed to fluoxetine on JNK activity. It is their understanding more pathways, other than just the JNK pathway are being activated, so they screened the cells not only on JNK activity, but also on activity of ERK and p38. As is already known, ERK and p38 are involved in the process of apoptosis. This has already been found in 1995 by Xia et al. They described the inverse relationship of p38 and ERK, in which both compounds increasing apoptosis (Xia et al., 1995). In fact, they also stated that JNK is correlated with p38 activity, for now this seems to be true and has yet to be confirmed by Mun et al. again.

For their research they used Hep3B cell cultures. These cultures were exposed to fluoxetine and using the technique of Western blot analysis, the activity of JNK, p38 and ERK were measured. The first result presented is the significant reduction of the number of cells within the cultures, as is shown in figure 12.



As figure 12 shows, the cell viability declines when higher amounts of fluoxetine are administered, leading to the first assumption that fluoxetine induces apoptosis (Mun et al., 2013).

Figure 12. Cell viability of Hep3B cells exposed to fluoxetine (Mun et al., 2013).

Afterwards, they analysed the activity of the three variables: JNK, p38 and ERK. These results are shown in figure 13.

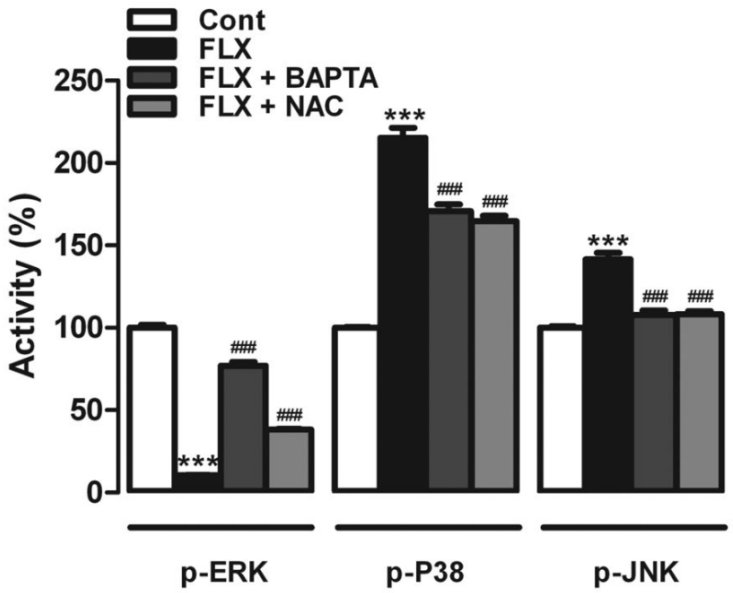


Figure 13. Shows the activity of the phosphorylated compounds ERK, p38 and JNK. It is shown that fluoxetine increases the activity of p38 and JNK and inhibits the activity of ERK. This confirms the relationship proposal of Xia et al. dating from 1995.

BAPTA and NAC were compounds antagonistic to fluoxetine, as it seems those compounds reduce the effect of fluoxetine, once again confirming the significant effect fluoxetine has (Mun et al., 2013).

Figure 13. The activity of ERK, p38 and JNK in cells exposed to fluoxetine and fluoxetine antagonists in Hep3B human hepatocellular carcinoma cells (Mun et al., 2013).

Mun et al. show in figure 13 how fluoxetine activates the JNK pathway. Considering the results from Chang et al. (figure 11) that JNK pathways induce apoptosis, it is once again confirmed that from the approach of the JNK pathway fluoxetine activate apoptosis. A useful addition is the fact fluoxetine increases p38, inducing apoptosis on its own, independently (Mun et al., 2013).

Controversial role of ERK

Finding lower levels of ERK in figure 13 is conflicting with the results shown in the Introduction to Ras-Raf-MEK-ERK, in which was found increased levels of ERK inhibit cancer cell proliferation. The results from figure 13 show the opposite. Because there are multiple compounds measured (cyclins and p53), it is hard to determine the precise effects of ERK alone. In Introduction to Ras-Raf-MEK-ERK is shown how activation of the Ras-Raf-MEK-ERK pathway leads to increased apoptosis, however, this could be a direct result of increased p53 and reduced amounts of cyclins (which was found as well) and not necessarily because of increased ERK levels, in fact, in that case increased ERK would even be undesired, but for some reason that effect was not strong enough to diminish the apoptosis inducing effect of p53. In figure 1 is presented ERK inhibits apoptosis (by inhibiting caspases), this seems to be the most plausible effect because this was found recently again, showing increased ERK levels promote tumour resistance to apoptosis (Liu et al., 2016). Moreover, ERK was found to have multiple purposes, for example in neurons where ERK activation leads to both apoptosis and proliferation (Li et al., 2014). In cancer, the controversial role of ERK was confirmed yet again, showing how ERK could be both tumour progressing and tumour suppressing (Deschênes-Simard et al., 2014). This pathway could function differently in different types of cancer. This means that in the case of Hep3B cells (figure 13) ERK is involved in apoptosis and in the case of figure 3 (undistinguished cell cultures) it works differently. One way or another, in figure 3 is shown how activation of the Ras-Raf-MEK-ERK pathway induces apoptosis, as already mentioned the most plausible cause for that is the increased amounts of p53 and the decreased amounts of cyclins, inducing both apoptosis and cell arrest.

In summary, the role of the ERK protein is not yet completely understood, knowing it could be both activate and inhibit apoptosis. However, it is shown that the Ras-Raf-MEK-ERK pathway consists of multiple processes and one of those is the increase of p53 and the decrease of cyclins. Activating this pathway is beneficial in those terms, not taking the function of ERK into consideration. Nevertheless, this should not immediately mean increased ERK levels are bad, because it is simply not completely understood yet what the exact function of ERK is.

Conclusion on SSRIs induced JNK activation

As figure 11 (Chang et al., 2008) and 13 (Mun et al., 2013) show, JNK activity inhibits proliferation of cancer cells and therefore slowing down the growth of the tumour. Firstly, in figure 11, this effect was introduced, and secondly, in figure 13, it is showed how SSRIs activate this process as well. This leads to the conclusion that SSRIs induce cancer cell apoptosis through the JNK pathway.

7. SSRIs managing T-cell activity involved in tumour growth

SSRIs interact with the immune system by inducing the release of cytokines by activating the immune system, leading from the fact macrophages produce TNF-α (Beutler, Cerami, 1989). Also it seems T-cells are activated by TNF-α (Beutler, Cerami, 1989). These findings demonstrate SSRIs activate directly macrophages and indirectly T-cells.

The reason why T-cells (and macrophages as well) are important in cancer is because they seem to play a role in antitumor activity. However, the exact role of T-cells in cancer is quite controversial. For example, one study finds that T-cells induce tumour progression (Jiang, Yan, 2016). In contrary, it has also been found that activation (T-cell proliferation) inhibits the growth of cancer (Grygier et al., 2013). These conflicting results suggest that T-cells have multiple purposes and could induce tumour growth inhibition by activating specific anti-tumour factors, demonstrating T-cells have various activities leading to different results, perhaps as well in different tissue types.

How do SSRIs trigger T-cell activity?

SSRIs, and especially fluoxetine, interact with T-cell activity. It is confirmed fluoxetine decreased T-cell activity (Pellegrino, Bayer, 2002), and this same result is found yet again, together with the observation fluoxetine manipulates T-cell activity without its SERT blocking, so through another pathway (Branco-de-Almeida et al., 2011). Other studies found SSRIs change T-cell proliferation by interacting with protein kinase C and cAMP levels (Edgar et al., 1999). Another study discovered fluoxetine suppresses T-cell proliferation by suppressing Ca^{2+} influx (Gobin et al., 2015).

The function of fluoxetine in tumours was investigated into and the results are shown in figure 14 (Frick et al., 2011).

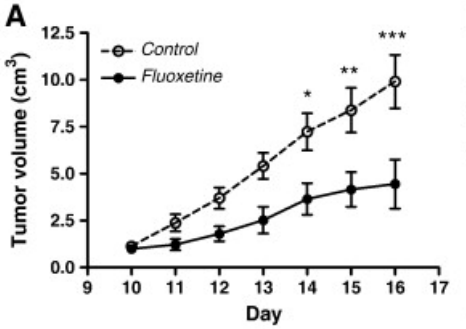


Figure 14 shows the result of the in vivo tumour growth of lymphoma tumour cells. It is showed fluoxetine significantly decreases the growing rate of tumours (Frick et al., 2011).

Figure 14. Tumour volume in lymphomas, in fluoxetine and in a control (Frick et al., 2011).

These results are not necessarily showing the T-cell activity. To confirm those, they performed measurements of mRNA expression of cytokines, shown in figure 15 (Frick et al., 2011).

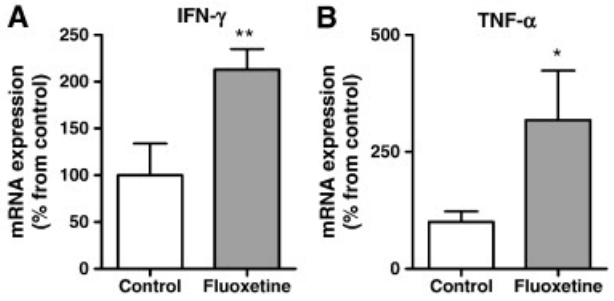


Figure 15. These charts show the results of mRNA expression to measure activity of the cytokines IFN-ɣ and TNF-α. It is shown that fluoxetine significantly increases the activity of both cytokines, compared to the control situations (Frick et al., 2011).

Figure 15. mRNA expression of IFN-ɣ and TNF-α (cytokines), (Frick et al., 2011).

Conclusion of SSRIs and immune system activity/T-cell activity

The results in figure 15 show the increase of cytokines induced by fluoxetine (Frick et al., 2011). This shows increased activity of the immune system. It still is the question how increased immune system activity interacts with tumours. As shown in figure 14 tumour growth is inhibited by fluoxetine and therefore these results are an indication immune system activity is tumour inhibiting (Frick et al., 2011), however, more research should be done, because it is not completely clear the inhibition of the tumours is a direct result of increased cytokine levels, or is induced by other pathways, for example the pathways examined in other sections.

Still, these results show involvement of the immune system and combining other research of Alvaro et al. lead to new insights. Alvaro et al. concludes there is a causal relationship between increased immune responses and tumour suppression in lymphoma cells (Alvaro et al., 2008).

Future research should include more types of cancer tissue to examine immune system proliferation in various cancer types and should investigate into the function of IFN-ɣ and TNF-α in cancer, including other cytokines, because they seem to be involved in cancer as well, for example IL-10 (Mannino et al., 2015), IL-11 (Putoczki et al., 2015) and TGF-ß (Fabregat et al., 2014). Additionally should be examined whether SSRIs increase or decrease levels of these other cytokines.

8. Selecting the right SSRIs within the group

As stated in Characteristics of SSRIs there are multiple approved SSRIs currently used and distributed as antidepressants. Until now only a few SSRIs were used in research; and most of the times these were fluoxetine and sertraline.

Despite SSRIs working through the same mechanism, there could be differences in function in targeting the desired pathways. The research group of Kuwahara et al. tried to examine differences in function between various SSRIs. They found how sertraline had the highest increase of caspase activity. They tested this in human hepatocellular carcinoma cells. This result is shown in figure 16 (Kuwahara et al., 2015).

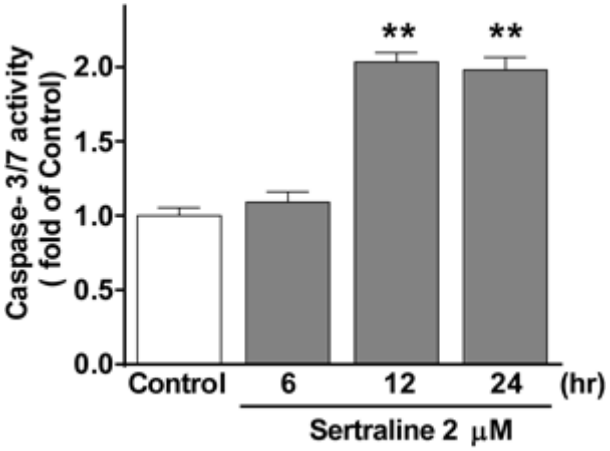


Figure 16. In this image is visualized what the effects are of 2 μM sertraline on the caspase activity in human hepatocellular carcinoma cells (in this case caspase-3 and caspase-7 activity).

It is shown that after 12 hours an increased caspase activity is found, after this 12 hour time span the activity does not increase and stays the same. Clearly sertraline has a significant impact on inducing caspase activity (Kuwahara et al., 2015).

Figure 16. Caspase activity in hepatocellular carcinoma cells in control cells and cells exposed to sertraline (Kuwahara et al., 2015).

Measuring other SSRIs

To measure the effect of a distributed drug they tested the cell viability (Kuwahara et al., 2015). The more apoptotic effect a drug has, the less cell viability will be measured. They expressed the cell viability in an IC_{50} (μM) concentration. This is the concentration drug needed for 50% inhibition of the desired effect in vitro (Stewart, Watson, 1983). The lower the IC_{50} value, the more effect the drug has. The results are visualized in table 1 (Kuwahara et al., 2015).

Table 1. IC_{50} values of cell viability of various SSRIs (Kuwahara et al., 2015).

SSRI name	IC_{50} value
Sertraline	1.24 ± 0.055
Paroxetine	7.34 ± 0.376
Fluvoxamine	31.0 ± 3.330
Escitalopram	94.8 ± 5.220

The results in table 1 show sertraline has the most apoptotic effect, followed by respectively paroxetine, fluvoxamine and escitalopram (Kuwahara et al., 2015). Sertraline is 75 times more powerful than escitalopram in inducing apoptosis and almost 6 times stronger than the number two paroxetine. Unfortunately, fluoxetine was not included in the research. Knowing the popularity of fluoxetine, future research should include fluoxetine. For now it can be stated sertraline has the most powerful apoptotic effect among the reviewed SSRIs (Kuwahara et al., 2015).

Discussion

The investigation into the function of SSRIs confirms how SSRIs target multiple, different pathways involving cell division, cell proliferation and cell apoptosis. From those findings it is clear SSRIs have a dual-purpose, besides of the classic antidepressant function, and this is not only the case in cancer, but also in other diseases like Alzheimer's disease, rheumatoid arthritis and multiple sclerosis. Its multifunctionality makes it an interesting concept to discover and the results show its potential.

From this perspective it shows how important neurotransmitter transmission is in the brain (and the rest of the body), and how (slight) alterations in those transmissions have effects on multiple processes in the body. Serotonin is along with dopamine, acetylcholine and epinephrine important in signal transmission in the brain and the CNS and therefore responsible for a great number of processes in the body. From this is clear that neurotransmitters have a great influence in the wellbeing and state of the body and its internal processes. By admitting drugs, or by manipulating these neurotransmitter levels in any way, it is clear the body will respond to that and in fact in more ways than intended or expected. This is confirmed by the list of side effects caused by SSRIs (Ferguson, 2001). People taking SSRIs to target their depression will most likely suffer from one or more of these side effects (for example nausea, dizziness, vomiting, weight gain, weight loss sexual dysfunctions or even anxiety) and for that reason taking SSRIs is not completely harmless.

From this follows the question how one should deal with the side effects experienced by cancer patients without depression. It is the goal to compare those side effects with the benefits SSRIs provide to cancer treatments. As is shown in the section combining TMZ with fluoxetine, it seems fluoxetine (and probably more SSRIs, as they work in a similar way) significantly enhances the function of chemotherapy and could therefore be a beneficial extension of the current cancer treatments. In this case fluoxetine and other SSRIs really function well in cancer treatments. In all diseases the side effects are always compared to the benefits, and knowing the aggressiveness and lethality of some tumours, cancer is on the far end of the spectrum, considering suffering. In these cases the side effects of SSRIs could be justified.

It has been shown how SSRIs target multiple pathways to induce cancer cell apoptosis or cancer cell arrest in the cell cycle by increasing caspase-9 and caspase-3 levels. This is shown in Conclusion on ERK, Conclusion on fluoxetine in brain tumours and Conclusion on SSRIs induced JNK activation. These sections show the effects of SSRIs and answer the main question of how SSRIs are involved in apoptotic cell pathways. Moreover, it is also shown in PC3 human prostate cancer cell that Ca^{2+} induces apoptosis and increasing concentrations of fluoxetine enhances that effect and in Conclusion of SSRIs and immune system activity/T-cell activity is shown fluoxetine significantly decreases the growing rate of lymphomas. A great benefit of these findings on SSRIs is that they pass the blood brain barrier (BBB) without trouble. As of now TMZ was one of the few chemotherapy compounds able to pass the BBB and with the addition of SSRIs that list of expanded.

Surely, question marks remain, because as is shown in Controversial role of ERK, the exact function of the ERK protein is not well understood. The conflicting results could not determine whether it is tumour promoting or tumour suppressing. However, its pathway, Ras-Raf-MEK-ERK induces apoptosis, caused by, partly or completely, increased p53 levels and decreased cyclin levels. To answer the question of in what extent ERK partly, or even does not, induces apoptosis, more research should be done on the function of ERK alone, in which other compounds (like p53) are not included. For now, activating the Ras-Raf-MEK-ERK pathway induces apoptosis, one way or another, and this should be researched various types of cancer tissue.

What should be done to use SSRIs in cancer?

This question is paradoxical because SSRIs are already being used in cancer, however not for the reason as described in this essay. Luckily, this provides the opportunity to examine its effect in cancer treatment, and as described in the section Depression and intaking of SSRIs in cancer patients, SSRIs do not influence cancer treatment negatively and therefore they are prescribed broadly to cancer patients suffering from a depression.

From combining TMZ with fluoxetine is clear how PET imaging provides information on the growth rate of tumours and from others sections follows how useful the techniques are of measuring apoptotic factors like cytochrome c, caspase-9, caspase-3 and PARP (poly (ADP-ribose) polymerase) and cell arresting compounds like p53 and p21. Closely monitoring the levels of these compounds in human tumour tissue exposed to SSRIs would provide valuable information about SSRIs use in human cancer.

Next phase of cancer research

Luckily, a great part of the complexity of intracellular pathways has been exposed and with that knowledge the next phase of cancer research can be entered. This means monitoring mentioned apoptotic factors, activity of the proteins ERK and levels of intracellular Ca^{2+} induced by the provision of SSRIs to (human) cancer tissue lead to new insights. These measures should be documented and are valuable for the complete concept of SSRIs use in cancer treatment. Again, SSRIs have a big advantage over classical cancer treatment because they are able to pass the blood brain barrier and its side effects have already been documented well, and moreover, they are globally approved as drugs. The overall results are promising.

Conclusion

SSRIs have effect on multiple intracellular pathways managing cell proliferation and cell apoptosis of cancer cells. These contain: inducing apoptosis through the Ras-Raf-MEK-ERK pathway, increasing Ca^{2+} influx and activating the JNK pathway. Furthermore, fluoxetine enhances chemotherapy treatments (in this case temozolomide) in brain tumours. Additionally, fluoxetine increases levels of cell arresting compounds, like p53, p38 and p21, apoptotic compounds like caspase-3, caspase-7 and caspase-9, and cytokines, such as IFN-ɣ and TNF-α.

SSRIs are already being used in cancer patients suffering from depressions, meaning its effects are known and there are found to be no conflictions between current cancer treatments and SSRIs admission. Besides of SSRIs inducing apoptosis and cell cycle arrest, they pass the blood brain barrier without trouble, making them access brain tumours.

Adding up these effects lead to the outcome that SSRIs have potential as cancer treatment or as an addition to current cancer treatments.

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