Cre loxP mouse models, light in the dark for ovarian cancer?

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Abstract:

Cancer is a growing problem because the average lifespan increases which raises the change to develop cancer. Due to medical limitations a lot of people die because of this disease. Ovarian cancer is a type of cancer with very poor prognosis due to the lack of understanding the etiology and few medical options. The biggest problem of chemotherapy nowadays is that they destroy almost the complete tumor but a few cells still survive resulting in resistance against the chemotherapy. A lot of methods have been used so far to study the onset and progression of ovarian cancer because there are many different subtypes. A method that mimics natural conditions of gene knockouts very closely is the Cre loxP system. It is a method to silence or activate genes of interest in only specific tissues. The use of Cre loxP in ovarian cancer has only started recently after it was proven to be very successful in the research of breast cancer. With this system it is possible to monitor the influence of certain gene knockouts or pathways. This thesis focuses on different mouse models that have been produced so far with Cre loxP in the research of ovarian cancer.

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1. Introduction:

1.1 Global impact of cancer

Because people tend to get older and older nowadays, the rate of cancer also increases. The chance of developing tumors is bigger at higher age. Also the way of living can contribute to a higher risk of developing cancer. Radiation and UV light are environmental factors that can damage DNA thereby resulting in cancer. Cancer can develop for instance when a tumor suppressor gene of the DNA in a cell mutates and therefore has no brake on dividing. There are also genes that promote cell proliferation. These genes are controlled very carefully and malfunction of the genes may lead to excessive cell growth, resulting in a tumor. Sometimes these genes mutate resulting in overly active cell proliferation. In most cases of cancer a series of mutations is necessary to develop a tumor as shown in figure 1. If a gene mutates of for instance DNA repair, more mutations are unrepaired, resulting in the loss of genetic information. This can in turn result in a mutation of a tumor suppressor gene resulting in excessive growth. Cancer usually develops after a cascade of several mutations as shown in figure 1. There are many different types of cancers because all kinds of cells can progress to tumor cells. Cells that need to replicate many times are more sensitive to becoming a cancer cell because in each cell division there is a chance that mutations take place. Although a lot of these mutations are repaired, there's always the possibility that a mutation is missed and remains unrepaired. If a cancer cell can evade apoptosis, a tumor develops. In many

cases the host doesn't notice anything of this growing tumor until the tumor has grown big and in many cases already has metastasized. Some cancers can be cured quite well already, where others are still almost incurable. Breast cancer nowadays has better prognoses than a brain tumor because it's quite easy to amputate or irradiate the breast (in combination with chemotherapy). The brain is not well accessible due to the skull and even if it's accessible, it's very dangerous to remove the tumor because critical areas of the brain can be damaged very easily. If metastasis has occurred chemotherapy is used to try to destroy the tumors throughout the body. Further in this article it will become clear that little is still known about ovarian cancers due to much different subtypes and different places of cancer origination. The aim of this paper is to present an overview of different ovarian cancers and mouse models that have been used so far.

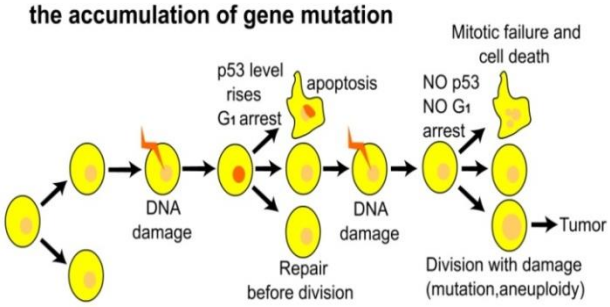

Figure 1: The figure shows that accumulation of gene mutations can contribute to the progression to tumor cells.
(http://jpkc.scu.edu.cn/ywwy/zbsw(E)/edetail12.htm)

1.2 Ovarian Cancer:

Studies have shown that ovulation increases the risk of developing ovarian cancer, while the oral contraceptive pill and pregnancy seem to reduce the risk. (Salehi, 2008) (Cramer, 2012). The ovarian surface epithelial cells are repeatedly exposed to inflammatory agents and mediators which can cause cellular and DNA damage which eventually can lead to malignant transformation (Fleming, 2006) (Riciardelli & Oehler, 2009) (R.J. & S., 2008). Ovarian cancers can be divided into subtypes because of different cell types. All these cells do not originate from a healthy ovary so it was believed that the development of the tumors was due to mullerian neometaplasia of the ovarian surface epithelium. As in all the cancers, tumors can be divided into malignant benign and intermediate (borderline). Malignant tumors are the main concern because they tend to proliferate rapidly and can cause stromal invasion, resulting in metastases in other parts of the body. Both benign and most of the borderline tumors lack the ability to invade underlying tissues. In rare cases a borderline tumor can evolve into a low-grade serous carcinoma (LGSC) which can cause stromal invasion. Although ovarian cancer is 10 times less common than breast cancer in percentage it results in more deaths due to poor prognoses. By using molecular genetic analysis, immuno histochemistry and histopathology, five main types of ovarian carcinomas have been identified: high-grade serous carcinomas (HGSCs; 70%), clear-cell carcinomas (CCC; 10%), endometrioid carcinomas (EC; 10%), Low-grade seroud carcinomas LGSC (<5%) and mucinous carcinomas (MC; 3%), and that are inherently different diseases (figure 2)

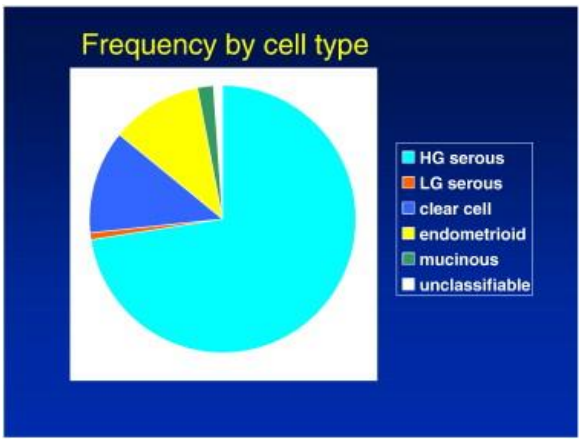

Figure 2: Figure shows the frequency of the main types of ovarian cancers (Blake Gilks & Prat, 2009).

All the subtypes respond differently to chemotherapy and have different prognosis. Light microscopy can be used for the diagnosis of ovarian carcinomas. Further in this article it will become clear that it's not even necessary that ovarian cancer originates from the ovary. The cancer cells can also originate from the fallopian tube (Blake Gilks & Prat, 2009) (Kim, Coffey, & Creighton, 2012).

1.3 High-grade serous carcinoma (HGSCs)

HGSCs are the most common ovarian carcinomas, they account for approximately 70% of the ovarian cancers (Figure 2). They have a wide morphologic spectrum within the subtype but their growth pattern is very distinctive. The cells have a very high proliferative rate, the size can vary widely. Abnormalities (mutations or deletions) of Brca1, Brca2 and TP53 are most common cause of HGSCs (Köbel, Hunstman, & Gilks, 2008). These Brca and TP53 genes are important for double-strand break repair (DSBR), therefore abnormalities result in DNA damage and thus chromosomal instability. Carcinomas developing due to Brca1 of Brca2 mutations are most of the time high-grade serous types. In two thirds of the HGSCs and also sometimes in endometrioid carcinoma the Estrogen receptor is expressed while almost every mucinous carcinoma and clear cell carcinoma lack expression of this receptor (Köbel, Kalloger, & Carrick, 2009). New treatments are needed for HGCSs, which currently have a poor prognosis. Platinum/taxane chemotherapy is effective to approximately 70-80% of the HGSCs but most of the time there will be a reactivation resulting in a not curable carcinoma. (Krieger, Eckstein, & Schneider, 2010) (Godwin, Meistert, & O'dwyer, 1992). Regrowth takes place because cancer stem cells survive the chemotherapy which are capable of growing a new tumor (Shafee, Smith, & Wei, 2008). Later in this article this problem will be discussed more detailed.

1.4 Low-grade serous carcinoma(LGSCs):

LGSCs are not so common, as mentioned before they only account for less than 5 % of the ovarian cancers (Figure 2). This type of cancer has better prognosis, due to slower onset. The distinction between HGSCs and LGSCs can be made by looking at the nuclei. Nuclei of LGSCs show uniformity while HGSCs show big variation between the nuclei. K-RAS and BRAF mutations are very common in LGSCs. HGSCs show signs of chromosomal instability, this is not the case in LGSCs (Singer, Kurman, & Chang, 2002).

1.5 Mucinous Carcinoma (MCs)

MCs account for only 3 % of the ovarian cancers (Figure 2). About 80% of the MCs are benign, the remaining part is most of the time a borderline tumor (Koonings, Campbell, & Mishell, 1989). The K-RAS mutation is very common in this type of ovarian cancer. In 10 to 15% of the MCs Her2 seems to be amplified. Her2 is a proto-oncogene and overexpression and/or amplification of this gene is associated with an aggressive form of cancer (McCluskey, Olive, & Starnbach, 2012). MCs show low response rates to current therapies so investigation with new chemotherapies is needed (Hess, A'Hern, & Nasiri, 2004).

1.6 Endometrioid carcinoma (ECs):

ECs account for approximately 10% of all the ovarian cancers (Figure 2). Most of the ECs occur around or after the menopause. 'ECs closely resemble their counterparts arising from the endometrium'. The genes that are most commonly mutated in ECs are the B-catenin and Pten gene (Obata, S.J., & Watson, 1998) (Pal, Permuth-Wey, & Krischer, 2005). ECs seem to have the most favorable prognosis compared to other ovarian cancer types (Gilks, Ionescu, & Kalloger, 2008).

1.7 Clear Cell carcinoma (CCCs):

CCCs account for approximately 10% of all the ovarian cancers. Compared to ECs, CCCs have a less favorable prognosis (Gilks, Ionescu, & Kalloger, 2008).
The Arid1A gene is frequently mutated in clear cell carcinomas (Jones, Wang, & Shih, 2010). Further little is still known about which other mutations can play a role in the progression to CCCs. No Brca1 abnormalities are shown and CCCs also lack chromosomal instability (Press J.Z. & Boyd, 2008).

1.8 Cisplatin-taxane as a chemotherapeutic drug

The cisplatin-taxane combination (cisplatin) is widely used as a chemotherapeutic drug, it can be used for the treatment of various types of cancer. A big amount of cisplatin's antitumor effect can be addressed to the binding to DNA in the nucleus of the cell. Cisplatin has nucleophilic sites that can be reactive with the imidazole rings of guanine and adenine. These bases are most accessible for cisplatin in the major groove of the double helix of the DNA (Yang & Wang, 1999).
When this reaction occurs, adducts are formed which in turn can create intra- or inter-strand cross-links. These crosslinks disturb the structure of the DNA thereby blocking transcription or replication of the DNA (Payet, Gaucheron, & Sip, 1993). This blockage of transcription or replication can result in DNA damage. If DNA damage accumulates in a cell, apoptosis is triggered. Apoptosis is a controlled pathway of the cell to destroy itself, also known as programmed cell death (Barry, Benhke, & Eastman, 1990). There are also indications that the biggest effect of cisplatin in triggering apoptosis can be accounted to protein damage. (Kruidering, van der Water, & Zhan, 1998)(Fuertes, Alonso, & Perez, 2002).
Although cisplatin has a positive effect on cancer treatment the major problem of the treatment is that resistance can occur. There are theories that the cisplatin brings the tumor down to a few cancer stem cells which are able to survive the chemotherapy. They start multiplying again, resulting in a complete chemo resistant tumor (Krieger, Eckstein, & Schneider, 2010) (Godwin, Meistert, & O'dwyer, 1992). Stem cells are important cells for providing new cells for repair or maintenance of tissue. They have better protection for damaging agents. When all the normal cancer cells die because of chemotherapy, only the stem cells survive and they are capable of growing back a new tumor (Shafee, Smith, & Wei, 2008).
It seems that resistant cells have two different methods of dealing with cisplatin: first, the cells block induction of apoptosis, second, the cells find a way to stop cisplatin reaching the DNA (Pérez, 1998) (Johnson, Ferry, & Hamilton, 1998) (Fuertes, Alonso, & Perez, 2002).
As this resistance is a big problem because the tumor gets insensitive to chemotherapy there is desperate need for new chemotherapeutic drugs/targeted drugs. For the introduction of new chemotherapeutic drugs a lot of research first has to be done on good animal models of cancer. In the next chapter the Cre loxP system will be discussed and it will become clear that this system opens new doors in the development of creating and testing new chemotherapeutic drugs on different kinds of cancer.

2. The Cre loxp system

2.1 Genetically modified animals:

In the past a lot of research has been done with genetically modified animals. There is for instance the systemic knockout. With this method one gene is or multiple genes are knocked out in the whole body of the animal. This often resulted in lethality because embryos died early in the development, making investigation impossible. Then there's also the fact that a systemic knockout influences the whole body, this is very inaccurate because in cancer genes are only knocked out in specific tissues. A new method which circumvented this problem is the Cre loxP method, which will be discussed hereafter. It wil become clear that

this method can be very useful in cancer research.

2.2 Introduction of the Cre loxP system:

Since Dr. Brian Sauer developed the Cre loxp system new doors are opened for investigation with site-specific recombination. He originally developed it for the activation of genes in transgenic animals (Sauber, 1987) (Sauer & Henderson, 1988). Transgenic animals are genetically modified animal that have manually adjusted DNA. Dr Jamey Marth later discovered that this Cre-lox system could also be used for the inactivation of genes in selected cell types of transgenic animals by deleting loxP-flanked chromosomal DNA sequences (Orban, Chui, & Marth, 1992). In 1994 Drs Rajewsky and Marth reported that this Cre loxP system could also be used for conditional gene targeting in vivo (Gu, Marth, & Orban, 1994). The function of specific genes could be investigated in specific cell types by looking at the consequences after the knockout was performed. Nowadays Cre-lox recombination is used worldwide for conditional gene targeting in vivo. The recombination technique makes studies of specific gene function in healthy and disease organisms possible. You can selectively knockout or activate genes of interest in only specific tissues. The combination technique is called 'Cre loxP' because both components Cre and loxP need to be present for DNA recombination. Cre is a protein coming from the Cre gene, which can catalyze recombination of loxP (locus of X-over P1) sequences. Specific binding sites on this loxP sequences make it possible for Cre to bind. Cre is only expressed in the tissue(s) of interest because it's placed behind a tissue specific promoter. After binding of Cre, recombination can occur in the DNA sequence between the loxP sites. The Cre proteins cut the DNA between the loxP sites and ligate or 'glue' the DNA strands back together with DNA ligase (see figure 3). Figure 3 also shows how a mouse expressing Cre and another with the gene of interest flanked by loxP sites are crossed. The offspring express both Cre and loxP sites resulting in silencing of the gene in only the specific tissue(s). If there are more genes that are flanked by loxP sites all these genes will be cut out off the DNA and the effects can be monitored (Sauer & Henderson, 1988) (Sauber, 1987). Nowadays the Cre LoxP system can be used in plants and animals but it originates from bacteria. LoxP were found in the P1 bacteriophages of a bacterial virus. The chance to find a loxP sequence randomly in a genome is virtually zero because it consists of 34 base pairs. This gives us the opportunity to insert loxP sites into plants and animals and cut DNA very precisely because there is almost zero chance that you would cut DNA somewhere else in the body. The DNA gets cut when Cre is present in the same tissue as the loxP sites. Both the components are built in the genomes of the mice.

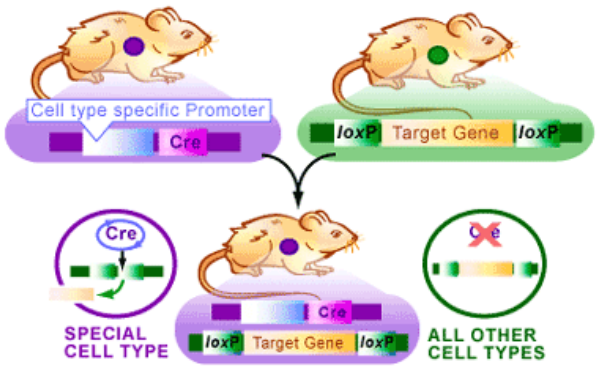

Figure 3 : One of the parents transcribes Cre in a certain tissue while the other parent has loxP sites surrounding the gene of interest. The offspring wil have both Cre and loxP, resulting in silencing of the gene in only one tissue. The Cre is expressed from the Cre genes which will in turn cut the loxP sites thereby silencing the target gene(s).

There are also other methods, for instance the injection of AdCre into the tissue of interest (Clark-knowles, Senterman, & Vanderhyden, 2009). The advantage of this method is that you can silence or active the gene at the precise moment you want it to happen by adjusting Cre manually. Before gene expression can take place the cellular machinery needs to be recruited to the gene of interest. The promoter on the DNA serves as a magnet for this cellular

machinery. After attachment to the promoter, the transcription of the gene behind the promoter can begin. Some genes in the body are always 'on' while others are only transcribed under certain conditions. For the Cre loxP system transgenic animals are necessary. Between the promoter and the gene of interest a stop codon surrounded by two loxP sites is inserted. Without the presence of Cre recombinase this gene will be turned off because the stop codon blocks the transcription of the total gene. In this way no mRNA transcript can be made so no proper working protein is produced. Figure 4 shows that in the presence of Cre recombinase the stopcodon will be excised and the gene can be transcribed. Cre loxP can also be used the other way around. Figure 5 shows that the Cre recombinase cuts out the gene of interest because it is flanked by two loxP sites. Like mentioned before the Cre loxP system can only be used with transgenic animals. First you need for instance a mouse with Cre recombinase in a specific tissue and you breed it with a mouse with loxP surrounding the gene(s) of interest. The offspring will lose the gene(s) because they have both Cre and LoxP sites (Sauber, 1987) (Sauer & Henderson, 1988) (Orban, Chui, & Marth, 1992).

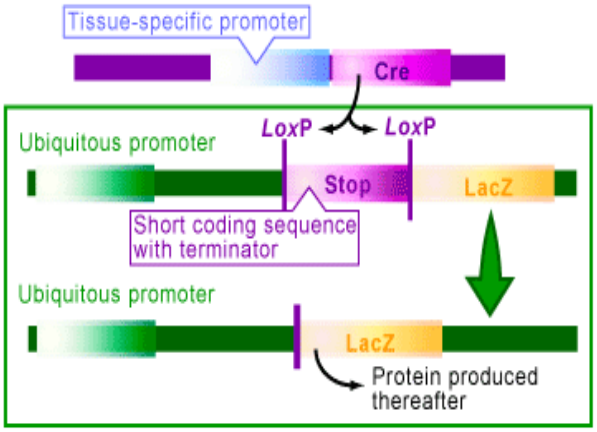

Figure 4 .: Cre attaches to the LoxP sites and excises the Loxp sites and stopcodon out of the DNA. After this process the promotor is attached to the LacZ gene and can be produced (Pechisker, 2004)..

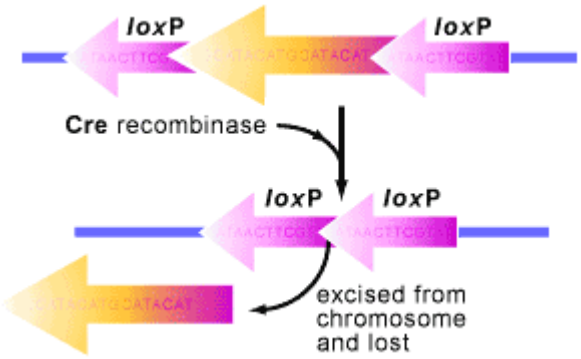

Figure 5 :This model shows how Cre recombinase binds to the loxP site and excises the sequence of DNA between the loxP sites (Pechisker, 2004).

3. Cre loxP in Cancer research

3.1 Cre loxP mouse models

Because of the Cre loxP system researchers have been able to develop mouse models for several types of cancer (Alberg, Lam, & Helzlsouer, 1999) (Paterson, 1998) (Flesken-Nikitin, Choi, & Eng, 2003) (Kim, Coffey, & Creighton, 2012). Also for ovarian cancer there are some mouse models created for the investigation of ovarian cancer.
Like mentioned before, the Cre loxP can silence or activate genes. The system can activate genes by removing the stop codon which is placed behind the promoter. Or the system can knock-out a gene by removing the exon between the two loxP sites.

3.2 Cre loxP in Breast cancer research

The Cre loxP system has already been used very intensively in the research of breast cancer. Because breast cancer is the leading cause of cancer and it affects about one in nine women in Western countries, so there is desperate need for new therapies to treat this type of cancer (Alberg, Lam, & Helzlsouer, 1999) (Paterson, 1998) (Kerr & Ashworth, 2001). Two important genes that have been silenced a lot with Cre loxP are: Brca1, Brca2 (Alberg, Lam, & Helzlsouer, 1999) (Paterson, 1998) (Kerr & Ashworth, 2001). Besides these genes there are

several other tumor suppressor genes, proto-oncogenes and other kinds of genes silenced/activated with the help of Cre loxP as cancer usually is a multifactor disease. Brca1 and Brca2 are under intensive study because mutations contribute to about 45% of the familial breast cancer. In most of the cases Brca1 or Brca2 has been mutated in combined familial breast/ovarian cancers . There is a direct link between Brca1 mediated breast and ovarian cancer (Alberg, Lam, & Helzlsouer, 1999) (Paterson, 1998). For a person who is a Brca1 mutation carrier the risk of developing breast cancer before the age of 70 is about 72%. For developing ovarian cancer this chance is about 40% (Brose, Rebbeck, & Calzone, 2002). Studies have shown that Brca1 mutated mice have impaired homologues recombination, growth retardation and defective DNA damage repair resulting in genetic instability. Homologues recombination is a very accurate way for a cell to repair DNA without losing genetic information (Deng, 2006)(Deng & Wang, 2003). Although Cre loxP has been used very intensively in breast cancer they only recently started with using Cre loxP in ovarium cancer.

4. Cre loxP in ovarian cancer research

4.1 The P53 and Rb genes

In 2003 **Flesken-Nikitin et** al chose to focus on two tumor suppressor genes, Rb and p53 Because a previous study shows that p53 mutations are common in sporadic ovarian tumors. Rb and p53 have interconnecting molecular pathways (Sherr & McCormick, 2002). Flesken-Nikitin et al used transgenic mice that possessed loxP sites flanking the Rb gene, the p53 gene or both of the genes. To cut the loxP site flanked genes they added AdCre into the ovary to monitor the influence of these gene knockouts. At a mean time of 227 days mice developed epithelial ovarian tumors in which both of the two genes p53 and Rb were inactivated. Tumor investigation led to the classification of either poorly or well differentiated serous neoplasms. In this case the Cre loxP system gives us a good opportunity to mimic the conditions of the development of ovarian cancer in women and monitor it very well. The results show that the pathology is quite the same and also the fact that the mice developed metastases in liver and lungs corresponding to human conditions. With this method the transformation of healthy cells into tumor cells can be monitored in a natural way. A striking conclusion was that only a very small percentage of the p53 knockout only and none of the Rb knockout only mice developed ovarian tumors. This shows that there might be a relationship between the molecular pathways of these two genes. Further investigation might give new insights into this relationship and may show new possibilities for treatment (Flesken-Nikitin, Choi, & Eng, 2003).

4.2 The K-RAS and PTEN genes

In the next study Dinulescu et al**.** examined the effect of the expression of the K-ras and Pten gene in the ovarian surface epithelium (OSE). Earlier studies have shown that Pten plays a key role in the etiology of endometrioid ovarian cancer. Apart from ovarian cancer, Pten mutations also play a role in the development of many other cancers. Pten is a tumor suppressor gene, it prevents cells from dividing, replicating and growing too rapidly. Another gene which can also be mutated in endometrioid ovarian cancer is the K-RAS gene. Mutation of K-RAS leads to a malfunction in normal tissue signaling and can be essential in the development of cancer. (Cuatrecasas, N., & E., 1998) (Gemignani, Schlaerth, & Bogomolniy, 2003). The transgenic mice used in the study possessed loxP sites flanking the Pten and the K-RAS gene. Recombinant adenoviral vector expressing Cre recombinase was delivered to the bursal cavity that encloses the cavity. Specific gene activation was achieved within the OSE. First they investigated the role of the K-RAS and Pten gene separately. K-RAS loxP (K-$ras^{G12D/+}$) mice showed signs of benign epithelial lesions and also endometriosis with no progression to ovarian carcinoma. In

endometriosis cells flourish and appear out of the uterus. $Pten^{loxP/loxP}$ mice showed no histological abnormalities in the ovaries. Then the investigators decided to test the PTEN and K-RAS mutation combination together in mice in the OSE. Every LSL-K-$ras^{g12D/+}Pten^{loxP/loxP}$ mouse developed invasive endometrioid ovarian carcinomas. The ovarian tumors were growing destructive infiltrative by replacing ovarian stroma, and originated from the OSE.

These are the first mouse models known for endometrioid adenocarcinoma and endometriosis. The biological behavior is mimicked very closely. This study raises new questions about the role of K-RAS mutations in human endomytriosis. Although the combination of K-RAS and PTEN mutations is tested in mice, this investigation gives reason to also explore the combination in human carcinomas. In conclusion, the results show that both the MAPK and the PI3K-AKT-mTOR-p70 s6K pathways are activated in the tumors in the LSL-K-$ras^{G12D/+}Pten^{loxP/loxP}$ mouse model. This raises the possibility to test the mice with specific inhibitors of these pathways like for instance PD 184352 and rapamycin. (Dinulescu, Ince, & Quade, 2005)

4.3 The Brca1, p53 and Rb genes

In this study Clark-Knowles et al. tested the conditional inactivation of Brca1, p53 and Rb in mouse ovaries. All the tumor suppressor genes were inactivated in certain combinations to study their roles in the transformation to ovarian cancer. Different mouse models were obtained by crossbreeding of mice possessing loxP sites around certain genes of interest. Cre was injected to the ovaries with intrabursal injection of AdCre. Like in the study mentioned before from Flesken-Nikitin et al they also used the p53 and Rb gene but in the investigators added the Brca1 gene. A Brca1 mutation can be a hereditary factor that accounts for approximately 10-15 % of ovarian cancers (Berchuck, Heron, & Carney, 1998) (Pal, Permuth-Wey, & Krischer, 2005).

In a large number of Brca1 mutated ovarian tumors also the p53 gene is mutated (Ramus, Bobrow, & Pharoah, 1999) (Zweemer, Shwa, & Verheijen, 1999). Like in their previous study they also examined the effect of a Brca1 mutation in mouse ovaries. This mutation resulted only in an increase of number of preneoplastic changes but no tumor formation (Clark-Knowles, Garson, & Jonkers, 2007). Even after one year of monitoring, the mutation in Rb gene alone gave no ovarian tumor formation at all. Combination of Brca1 and Rb deficiency also did not lead to tumor formation in the mouse ovaries. With the p53 gene deficiency alone, tumor formation was observed. Tumor formation was also observed in the p53/Rb, Brca1/p53 and p53/Brca1/Rb combinations. These data show that in all the cases p53 seems to be involved in the tumor formation. Clark-Knowles also investigated the sensitivity to cisplatin of the different ovarian cancers. Brca1, Rb and p53 mutations alone showed an increased sensitivity to cisplatin while a combination of two or even three genes showed no increased sensitivity at all.

All the mice with p53 inactivation developed invasive tumors in the ovaries. Brca1 inactivation in combination with p53 inactivation accelerated the tumor development (Clark-knowles, Senterman, & Vanderhyden, 2009).

4.4 The Wnt/B-Catenin and pI3K/Pten signaling Pathways

In this study from Wu et al. the main aim was to take a look at characteristics of the ovarian endometrioid carcinoma (OEAs). Because the investigators think it's more informative to know which types of mutations are likely to be found in a specific kind of ovarian tumor. With these results specific drugs could be developed that inhibit specific kinds of genes, pathways or other factors that help developing cancer. After serous carcinomas, ovarian endometrioid adenocarcinomas are the most common ovarian carcinoma. After comparing results of different studies they found a significant

correlation between the PI3K/Pten and Wnt/B-catenin pathway. They decided to investigate their role in ovarian cancer with Cre loxP. They tried to mimic human conditions with similar gene expression profiles and histological features with comparable PI3K/Pten and Wnt/B-cat pathway defects. One of the two ovaries was injected with AdCre while the other was left unharmed. All $Apc^{loxp/loxp}Pten^{loxp/loxp}$ injected ovaries developed tumors while the uninjected ovaries showed no signs for tumors at all. The study mentioned before from Dinulescu et al had results of tumor formation with activating K-RAS and inactivating PTEN the gene (Dinulescu, Ince, & Quade, 2005). Wu et al show in the data that a combination of K-RAS and PTEN is not very likely in ovarian cancers. A difference between both studies is that Dinulescu et al also make notice of endometriosis, while Wu et al have no results with endometriosis at all. Although the results show that both pathways might have cooperating mechanisms, their role in cancer pathogenesis is still unclear (Wu, Hendrix-Lucas, & R., 2007). Review of different studies show contradicting results. Some studies suggest that the pathways are not mutually exclusive while others suggest that the PI3K/Pten and Wnt/B-cat pathways are different (Mulholland, Dedhar, & Wu, 2006).

4.5 Pten and Dicer knockout

In this study Kim et al used the double knockout of the Dicer and Pten genes. The Dicer gene is essential for microRNA synthesis. The Pten gene is a tumor suppressor gene which inhibits the PI3K pathway. Conditional knockout was obtained by using loxp sites flanking the genes of interest and disabled with Amhr2-Cre. Amhr2 is used for promoter dependant Cre expression. This is anti-Müllerian hormone receptor type 2-directed Cre which is expressed in the mullerian duct. All ovaries subjected to Cre developed ovarian cancer followed by death due to metastases. Pten or Dicer gene knockout alone did not lead to tumor formation at all. The tumors seem to arise from the fallopian tube thereby supporting the "fallopian tube hypothesis". The cancer spreads from the fallopian tube into the ovary with aggressive metastases to the dominal cavity. The developing tumors match the characteristics of high-grade metastatic serous carcinomas. So this study provides evidence that serous ovarian carcinoma arise from the fallopian tube. To support this evidence they decided to further test this fallopian tube origin. In a group of mice they removed one ovary to check whether they would still develop ovarian cancer. After removal of the ovary, tumor formation was still observed. In another group of mice they removed the fallopian tubes, in this group no tumor formation was documented. Taking all these data together the scientists provide evidence that serous ovarian carcinoma arise from the fallopian tube (Kim, Coffey, & Creighton, 2012).

5. Conclusion/Discussion:

It is clear that the Cre loxp system has opened a lot of doors for the research in ovarian cancer. Before this system there weren't any accurate ways to properly investigate the onset and progression of ovarian cancer in such a natural way. Systemic knock outs often resulted in lethality or unnatural conditions. Cre loxp is quite a good method to mimic normal conditions of the progression to ovarian cancer. It also gave rise to the opportunity to test new chemotherapeutics in the genetically modified animals. This is very important for the treatment of ovarian cancer because there are so many types. The different types do not originate all from the same tissue. Monitoring the progression of ovarian cancer in genetically modified animals created the possibility to behold the development of the different types of ovarian cancer very accurately. Each type of ovarian cancer has its own genes that used to be mutated. As mentioned before it's possible to selectively mark genes of interest with loxP sites and monitor the influence of the mutations.

The difference between the natural way of the progression to ovarian cancer is that you have a series of mutations that eventually can give rise to ovarian cancer. As shown in figure 1 the mutations take place after each other. A lot of time can pass before the next gene mutates. With the Cre loxp method all the genes of interest are knocked out at once, there's a possibility that the outcomes won't be totally the same.

A disadvantage of this method is that it takes quite some time before you have the proper mouse models. There are many types of genes that can be mutated so it is also very time consuming to test each combination of knockouts. That is why it is good to start with the combinations of mutations that seem to exist in most types of ovarian cancer. As mentioned before the different ovarian cancer types respond differently to chemotherapeutics. Chemotherapeutics can eventually be tested on each Cre loxp mouse model. In the future this might lead to 'personal medication' for each type of ovarian cancer. A sample can then be taken from the tumor to analyze which pathways are influenced and what genes are mutated. Microarrays can take it one step further because a lot of molecular information can be extracted. All the samples can be integrated to find patterns in types of cancers. If you combine all this information it is possible to classify tumors and discover therapeutic markers. With this information, certain chemotherapeutics can be tested to eventually be able to take the best therapeutic agent for a certain subtype of cancer (Perez-Diez, Morgun, & Shulzhenko, 2007). However when you get to more sophisticated ways to treat cancer, for instance monoclonal antibody therapy the problem arises that the antibodies will not react the same in humans as they do in mice. Because antibodies most of the time don't bind to the mouse receptor while they do bind to the human receptor. The human immune system differs with the immune system of a mouse. That is why studies performed in mice might not give the exact same outcome as in humans. In the Cre loxp knockout method this is a smaller problem because they show great similarities between the two genomes. Many of the genes are conserved during evolution. Although the Cre loxP system can be time consuming it is the best way we have so far and it has helped us understand the onset and progression of ovarian cancer a lot better.

Works Cited

Alberg, A., Lam, A., & Helzlsouer, K. (1999). Epidemiology, prevention, and early detection of breast cancer. *Current opinion in oncology*, 11:435-441.

Barry, M., Benhke, C., & Eastman, A. (1990). Activation of programmed cell death (apoptosis) by cisplatin, other anticancer drugs, toxins and hyperthermia. *Biochemica pharmacology*, 40: 2353-2362.

Berchuck, A., Heron, K., & Carney, M. (1998). Frequency of germline and somatic Brca1 mutations in ovarian cancer. . *Clinical cancer research*, 4: 2433-2437.

Blake Gilks, M., & Prat, J. (2009). Ovarian carcinoma pathology and genetics: recent advances. *Human pathology*, 40: 1213-1223.

Brose, M., Rebbeck, T., & Calzone, K. (2002). Cancer Risk Estimates for BRCA1 Mutation Carriers Identified in a Risk Evaluation Program. *JNCI journal of the National Cancer Institute*, 18: 1365-1372.

Clark-Knowles, K., Garson, K., & Jonkers, J. (2007). Conditional inactivation of Brca1 in the mouse ovarian surface epithelium results in an increase in preneoplastic changes. *Experimental cell research*, 313: 133-145.

Clark-knowles, K., Senterman, O., & Vanderhyden, B. (2009). Conditional inactivation of Brca1, p53 and Rb in mouse ovaries results in the development of leiomyosarcomas. *PLoS One*, 4(12): e85344.

Cramer, D. (2012). The epidemiology of endometrial and ovarian cancer. *Hematol oncol Clin North Am*, 26:1-12.

Cuatrecasas, M., N., E., & E., M. (1998). K-ras muations in nonmucinous ovarian epithelial tumors. *Cancer*, 82: 1088-1095.

Deng, C. (2006). Brca1: cell cycle checkpoint, genetic instability DNA damage respons and cancer evolution. . *Nucleic acids research*, 34: 1416-1426.

Deng, C., & Wang, R. (2003). Roles of Brca1 in DNA damage repair: a link between development and cancer. *Human molecular genetics*, 12: R113-R123.

Dinulescu, D., Ince, T., & Quade, B. (2005). Role of K-ras and Pten in the development of mouse models of endometriosis and endometrioid ovarian cancer. *Nature medicine*, 11: 63-70.

Easton, D., Ford, D., & Bishop, D. (1995). Breast and ovarian cancer incidence in Brca1-mutation carriers. *American journal of human genetics*, 56: 265-271.

Fleming, J. (2006). Incessant ovulation, inflammation and epithelial ovarian carcinogenesis: Revisiting old hypothesis. *Mol Cell Endocrinol*, 247:4-21.

Flesken-Nikitin, A., Choi, K., & Eng, J. (2003). Induction of carcinogenesis by concurrent inactivation of p53 and Rb1 in the mouse ovarian surface epithelium. . *Cancer Research*, 63: 3459-3463.

Fuertes, M., Alonso, C., & Perez, J. (2002). Biochemical modulation of Cisplatin Mechanisms of action: Enhancement of antitumor activity and circumvention of drug resistance. . *Chemical reviews*, 103: number 3.

Gemignani, M., Schlaerth, A., & Bogomolniy, F. (2003). Role of KRAS and BRAF gene mutations in mucinous ovarian carcinoma. *Gynecologic oncology*, 90: 378-381.

Gilks, C., Ionescu, D., & Kalloger, S. (2008). Tumor cell type can be reproducibly diagnosed and is of independent significance in patients with maximally debulked ovarian carcinoma. *Human pathology*, 39: 1239-1251.

Godwin, A., Meistert, A., & O'dwyer, P. (1992). High resisance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. . *Biochemistry*, 89: 3070-3074.

Gu, H., Marth, J., & Orban, P. (1994). Deletion of the DNA polymerase beta gene in T cells using tissue-specific gene targeting. *Science*, 265: 103-106.

Hess, V., A'Hern, R., & Nasiri, N. (2004). Mucinous epithelial ovarian cancer: a separate entity requiring specific treatment. . *Journal of clinical oncology*, 22: 1040-1044.

Johnson, S., Ferry, K., & Hamilton, T. (1998). Recent insights into platinum drug resistance in cancer. *Drug resistance updates*, 1: 243-254.

Jones, S., Wang, T., & Shih, L. (2010). Frequent Mutations of Chromatin Remodeling Gene ARID1A in Ovarian Clear Cell Carcinoma. *Science*, 330: 228-231.

Kerr, P., & Ashworth, A. (2001). New complexities for Brca1 and Brca2. *Current biology*, 11: R668-R676.

Kim, J., Coffey, D., & Creighton, C. (2012). High-grade serous ovarian cancer arises from fallopian tube in a mouse model. *PNAS*, Vol 109 10:3921-3926.

Köbel, M., Hunstman, D., & Gilks, C. (2008). Critical molecular abnormalities in high-grade serous carcinoma of the ovary. *Expert reviews in molecular medicine*, 10: e22.

Köbel, M., Kalloger, S., & Carrick, J. (2009). A limited panel of immunomarkers can reliably distinguish between clear cell and high-grade serous carcinoma of the ovary. *American journal of surgical pathology*, 33: 14-21.

Koonings, P., Campbell, K., & Mishell, D. (1989). Relative frequency of primary ovarian neoplasms: a ten year review. *Obstetrics and gynecology*, 74: 912-926.

Krieger, M., Eckstein, N., & Schneider, V. (2010). Overcoming cisplatin resistance of ovarian cancer cells by targeted lipsomes in vitro. *International journal of pharmaceutics*, 389: 10-17.

Kruidering, M., van der Water, B., & Zhan, Y. (1998). Cisplatin effects on F-actin and matrix proteins precede renal tubular cell detachment and apoptosis in vitro. *Cell death and differentiation*, 5: 601-614.

McCluskey, A., Olive, A., & Starnbach, M. (2012). Targeting HER2-positive cancer cells with receptor-redirected anthrax protective antigen. *Molecular oncology*, Online article.

Mulholland, D., Dedhar, S., & Wu, H. (2006). Pten and GSK3beta: Key regulators of progression to androgen-independent prostate cancer. . *Oncogene*, 25: 329-337.

Obata, K., S.J., M., & Watson, R. (1998). Frequent PTEN/MMAC mutations in endometrioid but not serous or mucinous epithelial ovarian tumors. *Cancer research*, 58: 2095-2097.

Orban, P., Chui, D., & Marth, J. (1992). Tissue- and site-specific recombination in transgenic mice. *Proc. Natl. Acad. Sci. USA*, 89: 6861-6865.

Pal, T., Permuth-Wey, J., & Krischer, J. (2005). Brca1 and Brca2 mutations account for a large proportion of ovarian carcinoma cases. . *Cancer*.

Palacios, J., & Gamallo, C. (1998). Mutations in the beta-catenin gene (CTNNB1) in deometrioid ovarian carcinomas. *Cancer research*, 58: 1344-1347.

Paterson, J. (1998). Brca1: A review of structure and putative functions. *Disease markers*, 13: 261-274.

Payet, D., Gaucheron, F., & Sip, M. (1993). Instability of the monofunctional adducts in cis-[Pt(NH3)2(N7-N-methyl-2-diazapyrenium)Cl](2+)-modified DNA: rates of cross-linking reactions in cis-platinum-modified DNA. *Nucleic acids research*, 21: 5846-5851.

Pechisker, A. (2004). TARGETING YOUR DNA WITH THE CRE/LOX SYSTEM. Online.

Pérez, R. (1998). Cellular and molecular determinants of cisplatin resistance. *European journal of cancer*, 34: 1535-1542.

Perez-Diez, A., Morgun, A., & Shulzhenko, N. (2007). Microarrays for cancer diagnosis and classification. *Advances in experimental medicine and biology*, 593: 74-85.

Press J.Z., d. L., & Boyd, N. (2008). Ovarian carcinomas with genetic and epigenetic Brca1 loss have distinct molecular abnormalities. *BMC cancer*, 8: 17.

R.J., K., & S., I. (2008). Pathogenesis of ovarian cancer: Lessons from morphology and molecular biology and their clinical implications. *Int J gynecol Pathol*, 27:151-160.

Ramus, S., Bobrow, L., & Pharoah, P. (1999). Increased frequency of TP53 mutations in Brca1 and Brca2 ovarian tumours. *Genes chromosomes cancer*, 25: 91-96.

Riciardelli, C., & Oehler, M. (2009). Diverse molecular pathways in ovarian cancer and their clinical significance. *Maturitas*, 62:270-275.

Salehi, F. (2008). Risk factors for ovarian cancer: An overview with emphasis on hormonal factors. *J Toxicol Environ Health B Crit rev*, 11:301-321.

Sauber, B. (1987). Functional expression of the Cre-Lox site-specific recombination system in the yeast. *Molecular cellular biology*, 7: 2087-2096.

Sauer, B., & Henderson, N. (1988). Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. *Proc Natl Acad Sci USA*, 85: 5166-5170.

Shafee, N., Smith, C., & Wei, S. (2008). Cancer stem cells contribute to cisplatin resistance in Brca1/p53-mediated mouse mammary tumors. *Cancer Research*, 68(9) : 3243-3250.

Sherr, D. J., & McCormick, F. (2002). The Rb and p53 pathways in cancer. *Cancer Cell*, 2: 103-112.

Singer, G., Kurman, R., & Chang, H. (2002). Diverse tumorigenic pathways in ovarian serous carcinoma. *The american journal of pathology*, 160: 1223-1228.

Struewing, J., Hartge, P., & Wacholder, S. (1997). The risk of cancer associated with specific mutations of Brca1 and Brca2 among Ashkenazi Jews. . *The new England journal of medicine*, 336: 1401-1408.

Wu, R., Hendrix-Lucas, N., & R., K. (2007). Mouse model of human ovarian endometrioid adenocarcinoma based on somatic defectsin the Wnt/B-catenin and PI3K/Pten siganling pathways. *Cancer Cell*, 11: 321-333.

Yang, X., & Wang, A. (1999). Structural studies of atom-specific anticancer drugs acting on DNA. *Pharmacology & therapeutics*, 83: 181-215.

Zweemer, R., Shwa, P., & Verheijen, R. (1999). Accumulation of p53 protein is frequent in ovarian cancers associated with Brca1 and Brca2 germline mutations. *Journal of clinical pathology*, 52: 372-375.