A close-up of colon cancer
Heijmans, Jarom

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Discussion
Conclusions, discussion and future perspectives

In the following section, two subjects of which the results have been shown in this thesis are discussed: the role of ER stress signaling in intestinal epithelial stem cell differentiation and the role of sex hormones in the development of colorectal tumorigenesis. All experimental chapters are discussed at the end of the chapter itself. In the discussion below, a number of questions are answered regarding how the experimental results relate to each other, to research performed by others and to disease. Additionally, future plans are discussed below and where possible, supported by preliminary data.

ER stress signaling in the intestinal epithelium

The molecular signals that impose the capacity for self-renewal on intestinal epithelial stem cells have received much attention. In contrast, little is known about pathways that reduce stemness and cause differentiation of stem cells into TA cells. Our studies on ER stress signaling in the intestinal epithelium reveal that levels of ER stress and downstream activation of the UPR are low in stem cells compared to TA cells. Activation of the UPR is both sufficient and necessary for intestinal epithelial stem cell differentiation and we demonstrate a key role for the ER transmembrane kinase *Perk* herein. Under normal circumstances Perk signaling contributes to stem cell differentiation and induction of high levels of ER stress causes stem cell depletion through activation of Perk.

Although it was previously described, that ER stress can cause cell cycle arrest by activation of Perk\(^1\), our results show that prior to loss of proliferation, the intestinal epithelium exhibits loss of stemness, thus exposing a distinction between stemness and proliferation per se. Additionally, whereas it was shown that blocking Perk signaling by enforced expression of a dominant negative variant of Perk (*dnPerk*) causes increased proliferation\(^2\), our results show additionally that stem cell numbers are increased upon stable knockdown of *Perk*. We conclude therefore that the activation status of the UPR marks both a descriptive and a functional distinction between stem cells and TA cells. The correlation between presence of ER stress and differentiation has previously been established\(^3\)-\(^5\). Our results assess that ER stress is present from the first step of differentiation, where a stem cell becomes a TA cell. Next to being correlated with differentiation, we observe a novel causal relationship: induction of ER stress is sufficient for differentiation.

We thus describe a novel function of the UPR. Likely, the UPR fulfills two roles in this function. The first may be an auxiliary role in the differentiation of stem cells. In the second role, the UPR may acts as a control mechanism that senses stress and pushes stressed cells out of the stem cell pool. Since many known stressors that induce ER stress also impair stem cell fitness, this mechanism may serve to protect the stem cell pool at the level of protein quality.
These data are the results of examining stem cell maintenance on an experimental level. It is however likely, that these findings are of direct consequence for pathological circumstances that are related to stem cells. Most notably diseases that are marked by stem cell loss and disease that are marked by an increased number of stem cells.

Perk signaling: pro- or antitumorigenic?
Our results show that Perk signaling causes loss of stem cells and that loss of Perk signaling causes stem cell accumulation under homeostatic conditions. These observations would predict that Perk harbors functions as a tumor suppressor gene. In contrast, it was previously shown that knockout of Perk from MMTV-Neu transformed breast epithelium resulted in reduced tumor growth. Since UPR functions are conserved between species and within species between tissues, it is unlikely that the function of Perk signaling in intestinal epithelium is opposite from that in mammary epithelium. Thus, Perk signaling, although it reduces stemness, is necessary for tumor growth, and may therefore be regarded as protumorigenic. These features are seemingly opposing characteristics of a molecular pathway may be explained by the fitness paradox.

Box 1
The Fitness Paradox

Certain signals that reduce stemness and eventually cause cell cycle arrest may do so to preserve cellular viability. In the case of ER stress and activated UPR signaling, cells that experience ER stress activate Perk signaling, which causes cell cycle arrest. In the absence of Perk, the effect of ER stress on the cell cycle is rescued, but cells eventually are more prone to necrosis or apoptosis due to proteotoxicity. The same may hold true for other mechanisms that do not involve UPR signaling: such as activity of P53 under a number of circumstances. Whereas irradiated cells may exit the cell cycle due to P53 activity, it was shown that in the absence of P53 myocardial cells succumb to necrosis upon irradiation. In these two examples, signals (such as Perk activity) that reduce stemness under physiological circumstances may do so to preserve cellular viability. Therefore, these signals may be regarded as a form of quality control. Blocking these signals may retain stemness against the cost of cellular fitness, resulting in cells that harbor exceeding sensitivity to all kinds of other stressors. It is as if cells would fail to realize that they are not suitable to maintain a stem cell position, resulting in a stem cell pool containing cells that may fail under circumstances that require high levels of fitness, such as during inflammation, nutrient deprivation or hypoxia. The fact that loss of a quality control gene causes increased stemness, but would result in reduced tumor growth is paradoxical. This paradox may be reconciled by what we call the fitness paradox. In the case of Perk
signaling, we show that loss of Perk, which renders cells insensitive to ER stress, preserves stemness. This occurs however against the cost of increased sensitivity to proteotoxicity. Eventually, this may result in cellular death, as was published in the initial report describing the function of Perk signalling. Thus, Perk signaling, although it reduces stemness, is necessary for tumor growth, and may therefore be regarded as protumorigenic. These seemingly opposing features of a molecular pathway may be explained by the fitness paradox.

Stressing the ER in disease, a druggable target?

A number of pathological conditions are marked by high levels of ER-stress. In the intestine, these include circumstances such as experimental epithelial damage, evoked by dextran sodium sulphate or in humans inflammatory bowel disease (IBD) such as ulcerative colitis and Crohns disease and ischaemia. Even though these conditions may or may not be elicited by alterations in ER stress signaling per se, it is likely that ER-stress induced stem cell loss contributes to the observed clinical image. Although lack of stem cells may not be considered the principal problem of these diseases, it may contribute to damage and prolong healing to an extent that is clinically of crucial importance.

In cancer, on the other hand, increased levels of ER-stress and high levels of the ER resident chaperone Grp78 protein have been described to correlate with bad prognosis. It seems contradictory that tumors have increased ER stress, since this would result in a decreased number of stem cells, which would cause a growth arrest of the tumor. Potentially, this contradiction is reconciled by the notion that tumors harbour so called cancer stem cells (CSCs), which may be excluded from the increased levels of ER stress observed. Additionally, whereas stem cells seem to be the cells of origin of intestinal tumors, cancer cells that have lost stemness may be able to revert to stem cells directed by signals from their niche. Therefore cancer cells that experience ER stress and lose stemness, may revert to CSCs after having reduced their ER stress levels. Increased levels of ER stress in tumors seem therefore not to be contradictory with carcinogenesis. Since in normal tissue ER-stress causes stem cell loss, it seems opportune to exploit this mechanism for the purpose of treating cancer. Cancer stem cells may harbor many mechanisms to elude chemotherapeutics. Additionally, cell cycle arrest upon induction of ER stress may be regulated – in part – by P53, a tumor suppressor gene often mutated in cancers. Preliminary experiments from our group show that CSCs may be sensitive to ER stress. Potentially, in analogy to normal epithelium, ER stress may differentiate CSCs, rendering them more sensitive to conventional chemotherapeutical agents. This approach may be promising in the treatment of chemotherapy resistant stem cells. Prior to implementing this treatment however, two problems have to be overcome: first, induction of ER stress may differentiate stem cells in healthy tissue, causing side effects known to many antiproliferative therapies such as several chemotherapeutical agents and
gamma irradiation. Second, cancer stem cells are known to evade chemotherapy by mechanisms such as expression of multi drug resistance pumps\textsuperscript{19}. These mechanisms may equally hamper delivery of ER stress inducing agents in cancer stem cells. Next to agents that induce ER stress, an increasing number of UPR modifying agents are described\textsuperscript{20-22}. These agents may protect cells from ER stress induced differentiation or apoptosis which could be of interest in disease such as IBD. Thus, whereas anti stem cell properties of ER stress signaling may be exploited to treat this
disease, these therapies will likely encounter similar problems as conventional cancer treatment, including adverse effects and difficulties in drug targeting due to drug resistance pumps.

**Altering the unfolded protein response in stem cell homeostasis**

Stem cells are recognized as critical players in development and disease. Identification of these cells and of signals that are important for stem cell homeostasis lead to better understanding stem cell biology and help spawn therapies manipulating these cells. Based on our results exposing a role for ER stress signaling in intestinal stem cell fate, we have initiated the analysis of the influence of ER stress signaling on other tissues. Additionally, we commenced to delineate UPR signaling in stem cell fate. Most notably, analysis of Ire1α and its downstream transcription factor Xbp1. In the following paragraphs, these two future lines of research will be elaborated and supported by preliminary results.

The intestinal epithelium is a model tissue for the investigation of stem cell biology. Stem cells are visible on microscope sections as crypt base columnar cells and stem cell hierarchy under homeostatic conditions is linear: stem cells differentiate into TA cells, which differentiate terminally, accompanied by movement of cells out of the crypt towards the villus. In the skin, stem cells were shown to come from a pool of slow cycling or quiescent cells that are localized in the bulge of the hair follicle. In most parts of the skin, keratinizing stratified epithelium is located in close proximity to these hair follicles, making it possible to feed from stem cells descending from the bulge. In other parts of the skin, such as the foot soles or hand palms, it is most logical that cells are derived from stem cells localized elsewhere, since hair follicles are not found in those tissues. In the gastrointestinal tract, a tissue with many similarities to such skin epithelium is found: the esophageal epithelium. In the esophagus, cells descend from proliferating cells that are localized in the basal layer. Likely, in this proliferative zone, both stem cells and TA cells reside. Hierarchy is difficult to distinguish since both cell types are localized in the same plane. Potentially, ER stress causes loss of stem cells to a similar extent in skin and esophageal epithelium as in the intestine. Induction of ER stress would facilitate the identification of those markers associated with stemness.

In order to investigate whether tissue self-renewal, and proliferation are affected in the esophagus, we analyzed esophageal tissue of mice that were injected with the ER-stress inducing agent thapsigargin (Tg). Analogous to the intestine, proliferation is reduced in the esophagus upon induction of ER stress (Figure 1A). Additionally, the most differentiated layer of keratinizing cells was expanded in mice that had received Tg compared to control animals, suggesting a shift from proliferation towards differentiation upon induction of ER stress. These results suggest that the differentiating role of ER stress signaling is not restricted to the intestinal epithelium, but may indeed be a generalized phenomenon.

Our results as outlined in chapter 3 reveal that in colorectal cancer cell lines, induction of ER stress causes a profound loss of stem cell markers and the stemness signature. We reasoned
Figure 2. Induction of high levels of active XBP1 causes ER stress and subsequent loss of proliferation. 
(A) Immunofluorescence of LS174T XBP1(s)TO cells, in which doxycyclin potently induces the presence of nuclear XBP1. (B) Induction of XBP1(s) causes cells to arrest in the G1 phase of the cell cycle, whereas S phase and G2 phase are reduced (Statistics were done using 2-way ANOVA followed by Bonferroni’s post test for multiple comparisons: *** P < 0.001). (C) Gene set enrichment analyses of LS174T cells in which XBP1(s) was induced for 20 hours. In the top panel, GSEA was performed in which the geneset consisted of the top 100 differentially upregulated genes upon SubAB treatment of LS174T cells, showing that XBP1(s) overexpression causes upregulation of ER-stress. In the middle panel, the geneset consisted of the top 100 downregulated genes from LS174T upon induction of a dominant negative TCF4, showing loss of the WNT-signature upon XBP1(s) induction. In the lower panel, a published intestinal epithelial stem cell signature as was obtained by comparison of Lgr5 hi vs. Lgr5 lo cells was used as geneset. This was shown to be de-enriched in cells in which XBP1(s) is activated. (D) Western blot analysis of LS174T XBP1(s)TO cells. Induction of XBP1(s) causes phosphorylation of eIF2α, upregulation of CHOP and loss of C-MYC. (E) Crystal violet staining of cells expressing XBP1(s) and constitutively activated Gadd34, shows that XBP1(s) induced cell cycle arrest is caused by phosphorylation of eIF2α.
that since intestinal stem cell markers are found between those genes that are most profoundly
downregulated in colorectal cancer cell lines, analysis of differentially downregulated genes from
esophageal cell lines would aid in the identification of esophageal stem cell markers. To that end
we treated two esophageal squamous cancer cell lines with the GRP78 cleaving enzyme Subtilase
AB toxin and performed RNA expression microarray analysis. Of all differentially downregulated
genes, we found a total of 47 genes to be lost in both cell lines upon induction of ER stress
(Figure 1B). Further expression analysis of these genes by means of mRNA in situ hybridization
may reveal distinct staining patterns in the basal layer of the esophagus, potentially leading to
better understanding of stem cell hierarchy in this tissue.

The two most conserved branches of the UPR, Perk signaling and Ire1α signaling reportedly
have opposing effects: Perk signaling causes a reduction in cell cycle progression and results in
apoptosis while Ire1α signaling increases proliferation. These divergent roles that emanate from
a common signal result in a balanced outcome. Therefore, loss of Ire1-Xbp1 signaling may result
in reduced stem cell numbers and increased signaling could cause stem cells accumulation. Ire1α
activates the transcription factor Xbp1. Additionally it plays a role as a JNK-kinase. Although
JNK is intricately involved in stem cell maintenance in the intestine, a role for Xbp1 in stem
cell homeostasis is less investigated. To analyze the function of Xbp1 in the intestinal epithelium,
we generated an LS174T colon cancer cell line that expresses the active form of Xbp1 (Xbp1(s))
under the control of a doxycyclin sensitive promoter (LS174T-Xbp1(s)TO) (Figure 2A). Surprisingly,
induction of high levels of Xbp1 caused these cells to arrest in the G1 phase of the cell cycle
(Figure 2B). To analyse the mechanism of cell cycle arrest in these cells, we performed mRNA
microarray analysis cells in which Xbp1(s) was induced and compared these to cells that were
untreated. Analysis of differentially expressed genes after induction of Xbp1(s) resulted in the
identification of a panel of known transcriptional Xbp1 target genes. Curiously, using gene set
enrichment analysis (GSEA) we found in these cells that induction of high levels of Xbp1(s) causes
loss of the stemness signature as defined in chapter 3.2. In addition, a list of 100 WNT target
genes, most significantly down regulated upon induction of a dominant negative form of TCF4 was
highly significantly de-enriched. GSEA of an ER stress signature (top 100 upregulated genes
derived from differential expression of LS174T cells in which ER stress was induced by SubAB,
chapter 3) showed enrichment, arguing that induction of high levels of XBP1(s) causes loss of
stemness, loss of WNT signaling and activation of ER stress signaling (Figure 2C). To analyze
whether the ER stress response as observed by induction of XBP1(s) is mainly due to transcription
of targets of XBP1(s) or whether this is the result of generalized UPR activation, accompanied by
activation of other UPR branches such as PERK, we analyzed the level of eIF2α phosphorylation.
Indeed, upon induction of XBP1(s), a generalized UPR is activated and eIF2α is phosphorylated
(Figure 2C). To analyze whether the cell cycle arrest upon induction of Xbp1(s) was caused by
Perk-eIF2α signaling, we reverted eIF2α phosphorylation by enforced expression of constitutively
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Continuous dephosphorylation of eIF2α rescued the phenotype of Xbp1(s) overexpression induced cell cycle arrest (Figure 2D). Together, these results show that induction of high level Xbp1(s) causes a generalized UPR, where eIF2α phosphorylation is induced. This in turn causes cell cycle arrest. Whether loss of stemness and loss of WNT signaling is also reverted by inhibition of eIF2α phosphorylation remains to be tested.

The notion that Xbp1(s) induces eIF2α phosphorylation suggests interaction between different branches of the UPR and experiments that show induction of the pro-apoptotic transcription factor Chop upon overexpression of Ire1α are supportive of these data33. Likely, Xbp1-eIF2α interaction is a result of high levels of Xbp1, since moderate levels of isolated Ire1α activation

Figure 7

Figure 3. Xbp1 targeting strategy.
To obtain the targeted Xbp1 knockin allele, the endogenous exon 4 and 5 were duplicated. The first exon 4 and 5 were fused and put before a strong stop signal (triple polyA signal). Subsequently, the whole of exon 4, 5 and the polyA signal were surrounded by loxP sites and put in front of a FRT-surrounded neomycin resistance cassette. The second exon 4 was replaced by a copy in which the 26 base pairs, present in the endogenous exon 4, was spliced out. In this fashion, depending on breeding strategies, two distinct mice can be made: 1 Xbp1 conditional knockout allele: Cre mediated deletion of the knockin allele yields a truncated (inactive) mRNA of Xbp1. 2 Xbp1 conditional active allele: FRT recombination of the knockin allele will put the spliced exon 4 behind the unspliced exon 4. This allele functions as a wild type allele, but cre-mediated recombination will excise the wild type (unspliced) exons 4 and 5. In this fashion, a spliced (active) mRNA will be transcribed from the endogenous locus.
in Hek293 cells do not induce translation of the Perk target Atf-4, deflecting absence of phosphorylated eIF2α. Possibly our results identify a bidirectional role for Xbp1 in which one level of expression may arrest cells in the cell cycle whereas another level of expression may be capable of causing hyperproliferation. Potentially this is linked to the level of protein production, since high levels of Xbp1 activity are associated with reduced protein production due to induction of degradation machinery, whereas low levels of Xbp1 activity are correlated to an increase in protein production. A similar role where levels of a gene decide between cell cycle progression and arrest has previously been shown for transcription factor c-Myc.

To genetically induce Xbp1 activation in moderate levels as achieved by the endogenous locus, we targeted the Xbp1 locus in such a fashion that facilitates both activation and inactivation of Xbp1, depending on a combination of breeding and cre-mediated recombination. Epithelium specific deletion or activation of Xbp1 will aid in the definitive identification of the role of Xbp1 signaling in stem cells (Figure 3).

To conclude, the notion that ER stress signaling causes intestinal stem cell differentiation may be true for more tissues then the intestinal epithelium and we propose to utilize ER stress as a mechanism to reduce stemness and identify a disappearing cell signature, that could be associated with progenitor cells in the esophageal epithelium. Furthermore, we propose to investigate downstream signaling of the UPR in intestinal stem cells. This in order to shed light on the question to what extent the UPR is involved in cell fate decisions and cellular fitness, two processes intricately involved in stem cell biology and tumorigenesis.

**Sex hormones in colorectal carcinogenesis**

In the second part of this thesis, the subject of sex hormones during CRC development is investigated. Women suffer less from CRC than men. In a large randomized controlled trial, it was found that women that receive hormone replacement therapy (HRT) containing conjugated estrogen and the progestin medroxyprogesterone acetate (MPA) have reduced prevalence of CRC, whereas estrogen monotherapy confers no protective effects. In our studies, we aim to set up an animal model for CRC that recapitulates these gender differences. We find that progestin signaling does not alter spontaneous tumorigenesis in animal models for development of CRC. We find no evidence for a protective role of female hormones in models of sporadic CRC. In contrast, gender disparity in sporadic CRC development seems to depend on tumor promoting effects that emanate from male gonads. Female hormones do exert a pro-inflammatory and pro-tumorigenic effect in colitis associated CRC (CAC), which is caused by estrogen signaling. Estrogens promote colitis and CAC through Erα, whereas estrogens promote CAC but not colitis through Erβ.

Although a role for sex hormones in CRC development was mainly suggested for the combination of estrogens and progestins, we did not find a role for progestin signaling in development.
op sporadic CRC in $Apc^{Min/\ast}$ mice, or in induction of the tumor precursor lesions ACFs, in rats using the alkylating agent azoxymethane (AOM). Potentially, differences between humans and rodents depend on the stage of carcinogenesis (polyps in $Apc^{Min/\ast}$ mice rarely progress beyond the adenoma stage and ACFs are the primary manifestation of the adenoma to carcinoma sequence).

The finding that female hormones, namely estrogens promote CAC is surprising. First of all, these data contrast the results obtained sporadic tumorigenesis in $Apc^{Min/\ast}$ mice, where ovariectomy was shown to increase tumor number$^{41}$. Secondly, whereas we find that estrogens contribute to inflammation, in a number of other organsystems such as the bone$^{42}$ and the liver estrogens were shown to be anti-inflammatory$^{43}$. In these organs estrogens were shown to inhibit production of IL6, a pro-inflammatory and pro-proliferative cytokine that is linked to intestinal tumorigenesis and increased in our model of CAC$^{44,45}$.

In the experimental model for colitis-associated cancer (CAC), addition of DSS to the drinking water causes colitis through direct damage to colonocytes. The epithelium responds to this damage with a wound repair response. Tumors, regarded as wounds that do not heal$^{46}$, will be smaller in size during circumstances of reduced wound repair and larger in size after increases in wound repair. Thus, in mouse experiments in which wound healing is reduced, a smaller tumor number is expected. This is in contrast to the human situation, in which severity and extent of colitis predicts the risk of developing CAC$^{47,48}$. This discrepancy results from the fact that in mice, DSS-colitis results from a physically damaging agent, to which the body reacts with wound repair. In humans, the damaging insult that the immune system delivers to one's own intestine may be regarded as a wound response itself.

Similar to what we observe in estrogen treatment, the combination of reduced sporadic tumorigenesis in $Apc^{Min/\ast}$ mice, but increased tumors in the DSS-AOM model was found in mice that lack the toll like receptor adaptor protein MyD88$^{49}$. Therefore, the mechanism by which estrogens promotes damage associated tumorigenesis potentially overlaps the conditions in MyD88 mice$^{49,50}$. In these mice, lack of MyD88 results in reduced wound repair upon an acute tissue damage evoked by DSS$^{50,51}$. Since later stages of wound repair are not inhibited, the degree of primary damage dictates the extent of late wound repair including hyperproliferation and presence of mitogens causing increased tumor growth in these animals. Since animals lacking Myd88 may have impaired primary but not secondary wound repair, these repair responses may be differentially regulated. The notion that mice lacking functional NFκB signaling deteriorate during colitis and have decreased CAC$^{52}$, argues a role for NFκB in both primary and secondary wound repair, but for MyD88 signaling predominantly in primary wound repair. Therefore, secondary wound repair may be mediated –in part- by non MyD88 related NFκB signaling, mediated through receptors such as the TNF receptor or through Ras-associated receptors. Further investigation of these damage responses may shed light on the exact regulation of these responses.
Which hormone to block?
Our experiments in rats point towards a pro-tumorigenic role for androgens in sporadic CRC development. In mice, in a model for damage associated CRC development, we find a pro-tumorigenic role for estrogens. Although clinical proof has been delivered that a combination of estrogens and progestins may exert protective effects in the development of sporadic CRC, a mechanism of action of these hormones is difficult to assess since animal models that exhibit similar effects are lacking (as shown in chapters 3.5 and 3.6). Additionally, despite a reduced incidence of CRC in women that received the aforementioned hormone combination, mortality in this group was similar to the placebo treated women, since tumors arising in the hormone treatment group were more aggressive and advanced\[^{38}\]. A shift from left sided to right sided tumors seems to occur upon treatment with female hormones. Potentially these data reflect protective properties on left-sided tumors, not right-sided tumors. Treatment with female hormones in postmenopausal women increases the risk of breast cancer and cardiovascular disease, rendering these hormones inappropriate for chemotherapeutic purposes\[^{37}\]. Potentially, female hormones could have a more restricted role in the secondary tertiary prevention of CRC, but more research is warranted. Blocking male hormones on the other hand may be investigated in more depth in animal models for CRC since little is known on how male hormones influence CRC development.

In the case of tumor promoting effects of estrogens on damage-associated cancer, judging from our results with estrogen monotherapy in \(Apc^{Pirc/+}\) rats and AOM injections, estrogens promote tumorigenesis exclusively in the presence of tissue damage. As described above, this is likely the result of increased tissue repair elicited by more severe colitis in the first place. It remains unclear whether the extent of tissue damage that is required to observe effects of estrogens is present during sporadic CRC development in humans. In case of colitis associated cancer development (CAC) in humans, this level of tissue damage may well be achieved. However, little is known on female hormones and IBD associated cancer\[^{53-56}\]. Prospective randomized studies in this field may shed light on a role of estrogen signaling in CAC development. For these studies, estrogen signaling would have to be blocked, posing a problem in a population of patients with IBD, comprising of women in their fertile age.

The future of hormone research in CRC
Based on our studies, three main research questions may be formulated. 1) \textit{How do female hormones influence CRC?} Although we find no clues that rodents may serve as a model to study protective effects of female hormones, further studies on human material may shed light on the mechanism of this protection. It has been noted that a combination of estrogens and progestins may cause this protection, but it is unclear whether progestin monotherapy may have similar effects. Furthermore, stratification of patients that have received MPA may result in more knowledge on which tumors exactly are prevented by hormone treatment. These studies could for example assess mutational status of tumor epithelium and immune cell infiltration.
2) How do male hormones promote tumorigenesis? Whereas our results clearly point towards tumor promoting properties of male hormones, the mechanisms by which this is caused remain elusive. A first step would be the identification of the hormone causing this effect. Secondly, since hormones may signal on every cell, generation of bone marrow chimeras of a receptor knockout allele may be a helpful step in this aspect. 3) What are the functions of the estrogen receptors in CAC and what is the effector cell? Our studies highlighting a role for estrogens in colitis associated tumorigenesis exposed a role for both the estrogen receptor α and β. Further studies may elaborate on the exact localization of the receptors that transduce these effects using bone marrow chimeras and on the effects of these receptors in the cell subsequently.

**Concluding remarks**

In the previous chapter, results were discussed on a pathway that reduces stemness and on gender disparity in colorectal cancer development. Both these lines of research are efforts to improve our understanding of the intestinal epithelium, of cells dividing in the crypt and of the development of malignancies from those cells. Despite efforts made by an enormous group of scientist, medical personnel and many more others, of which the writer of this thesis has been a minute part for the past five years, colon cancer remains one of the most life threatening dangers that looms over us. Performing experiments like these are contributions, however small, to an increasing body of knowledge, which is required for better understanding and treating of this disease.
7 Reference list


