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## Abbreviations

| Abbreviation | Term                        |
|--------------|-----------------------------|
| RPF          | Ribosome Protected Fragment |

## 1. Introduction

### 1.1. Cancer

According to data from the World Cancer Research Fund, in 2018 there were an estimated 18 million cancer cases worldwide of which 9.5 in men and 8.5 in women. Lung and breast were the most common cancers overall. However, the most common cancers among women were breast, colorectal and lung whereas for men these were lung, prostate and colorectal cancer.

Cancer is a disease in which cells start growing abnormally and evade mechanisms monitoring cellular integrity. Hanahan et al. (Hanahan & Weinberg, 2011) summarised such mechanisms and how cancer evades them as the hallmarks of cancer. These hallmarks will be discussed in section xxx.

In this thesis I will discuss two studies in which breast cancer and pancreatic cancer play a central role. Therefore in the next two sections will focus on these two very different cancers.

**1.1.1. Breastcancer** some text ater the subsection. some more text after the subsection. some text ater the subsection. some more text after the subsection. some text ater the subsection. some more text after the subsection.

**1.1.2. Pancreatic cancer** stats and explanation on pancreatic cancer

**1.1.3. Hallmarks of cancer** Hallmarks of cancer and lead into gene expression

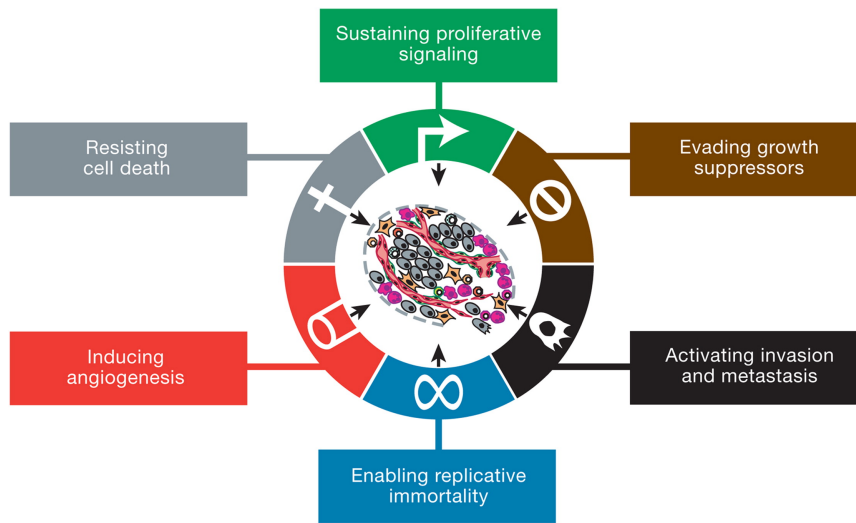


Figure 1.1: **\*\*The Hallmarks of Cancer\*\*** - This illustration encompasses the six hallmark capabilities originally proposed in our 2000 perspective. The past decade has witnessed remarkable progress toward understanding the mechanistic underpinnings of each hallmark. Reprinted from *Hallmarks of cancer: The next generation*, 144, Hanahan, Douglas and Weinberg, Robert A., *Hallmarks of cancer: The next generation*, 646-674, Copyright (2011), with permission from Elsevier

## 1.2. Central dogma of gene expression

### 1.3. mRNA translation

For the vast majority of protein coding mRNAs, eukaryotic mRNA translation occurs in the cytoplasm, however a small subset of mRNAs is translated in the mitochondria. mRNA translation is a process that includes initiation, elongation, termination and ribosome recycling and is an essential process. mRNA translation initiation is commonly regarded as the rate limiting step. Nevertheless, regulation of mRNA translation is also regulated at the elongation and termination phases to a lesser extent.

**1.3.1. mRNA translation initiation** In eukaryotes most mRNAs are translated by scanning the mRNA for a start codon (AUG). This mechanism begins with the formation of the 43s pre-initiation complex (PIC) consisting

of methionyl-initiator tRNA (met-tRNA<sub>i</sub>) in a ternary complex (TC) with guanosine triphosphate (GTP) bound eukaryotic initiation factor 2 (eIF2). The PIC is recruited to the 5'-cap of mRNAs which is facilitated by the eIF4F 5'-cap binding complex, a complex consisting of eIF4E (cap binding protein), eIF4A (RNA helicase) and eIF4G (scaffolding protein). The PIC then scans along the mRNA from the 5' end until it encounters an AUG codon. After AUG recognition eIF2-GTP is hydrolyzed forming a stable 48S PIC. After release of eIF2-GTP the 60S ribosomal subunit joins to form the 80S ribosome and protein synthesis can commence (10, 11). Next to this scanning mechanism, mRNA translation can also be initiated via alternative cap independent mechanisms (12).

**1.3.2. mRNA translation elongation** The 80S ribosome contains three sites; the acceptor (A), peptidyl (P) and Exit (E) sites. After initiation, the 80S ribosome is positioned with the met-tRNA<sub>i</sub> in the P site at the AUG codon with the following codon of the transcript in the A site awaiting its cognate tRNA. The tRNA arrives in a TC together with eukaryotic elongation factor 1A (eEF1A) at the A-site of the ribosome. After arrival in the A-site, the codon is then recognized. The binding of eEF1A is GTP dependent, recognition of the cognate codon by the tRNA triggers hydrolysis whereby eEF1A releases from the tRNA that is then recycled by eEF1B. Peptide bonds are then formed accompanied by a tRNA hybrid state whereby acceptor sites of tRNAs in the A- and P-site now move to the P- and E-site. Binding of eEF2-GTP then promotes translocation of the tRNAs into the P- and E-sites after which eEF2B-GDP releases. After release of the deacylated tRNA from the E-site the ribosome is ready for the next cycle. This process is repeated until a stop codon (UAA, UGA or UAG) is detected by the ribosome (11). mRNA translation termination Termination of mRNA translation is facilitated by two release factors, eRF2 and eRF3-GTP. The TC with eRF2 and eRF3-GTP binds to the A-site of the ribosome. Recognition of the stop codon by the ribosome

then causes hydrolysis resulting in a conformational change and release of the polypeptide. eRF1 and the ATP binding cassette protein (ABCE1) together promote the splitting of the 60S and 40S subunits, of which the 40S subunits has still bound tRNA. After release of the tRNA from the 40S subunits the parts of the translational machinery can be recycled(11).

#### **1.4. Regulation of mRNA translation**

**1.4.1. mTOR signalling pathway** mTOR is a conserved Ser/Thr kinase and is found in two structurally and functionally distinct complexes, mTORC1 and mTORC2. mTORC1 contains mTOR, regulatory associated protein of TOR (raptor), the GTPase beta-subunit like protein (GbetaL) and disheveled, EGL-10, pleckstrin [DEP] domain containing mTOR-interacting protein (depor). mLST8 and depor are found in both mTORC1 and mTORC2. However, rapamycin-insensitive companion of TOR (rictor), mammalian stress-activated protein kinase [SAPK]-interacting protein (mSIN1), and proline-rich protein 5 (protor) are specific to mTORC2(14, 15). In regards to regulation of mRNA translation, mTORC1 is a key player in regulation of translation initiation through facilitating the release of eIF4E from 4E-BPs via phosphorylation of 4E-BPs by mTOR (16). Furthermore, substrates of mTORC1 include ribosomal S6 kinases (S6Ks) 1 and 2(17), and La ribonucleoprotein domain family member 1 (LARP1)(18). mTORC2 is found to associate with ribosomes to promote co-translational phosphorylation and folding of nascent Akt polypeptide(19). As mentioned mTORC1 is activated via growth hormones including insulin and insulin like growth factor (IGF). For example, when insulin binds to the insulin receptor, tyrosine kinases (RTKs) and phosphoinositide 3-kinase (PI3K) are activated. Phosphatidylinositol 3,4,5-triphosphate (PIP3) is then generated by PI3K from Phosphatidylinositol 4,5-bisphosphate (PIP2). This step is reversed by PTEN which hydrolyzes PIP3 to PIP2, thereby working antagonistically to PI3K. PIP3 recruits AKT and phosphoinositide-dependent kinase 1 (PDK1) towards

the plasma membrane where AKT is phosphorylated by PDK1. Ras homologue enriched in brain (Rheb) is a GTPase that stimulates mTOR in its GTP bound form. The tuberous sclerosis complex (TSC) consists of TSC1 (scaffold protein) and TSC2 is a GTPase-activating protein (GAP) which inhibits Rheb through hydrolysis of Rheb-GTP to Rheb-GDP, thereby inhibiting mTOR activity. AKT mediates phosphorylation of TSC2, leading to a decreased GAP activity and reduced mTOR inhibition. Signaling through the Ras GTPase by growth factors may also activate mTORC1 through the RAF/MEK/ERK axis whereby extracellular signal-regulated kinase (ERK) leads to direct phosphorylation of TSC2 and raptor or via the RSKs(6, 20, 21). Protein synthesis is the most energy expensive process within cells(22). Therefore, regulation of mRNA translation is tied to cellular energy levels. AMP-activated protein kinase (AMPK) is a kinase activated by increased AMP/ATP ratios as well as ADP/ATP ratios. AMPK inhibits protein synthesis by activation of TSC2, thereby reducing mTOR activity. Furthermore, cellular oxygen levels are linked to ATP production, where low oxygen levels reduce ATP production leading to AMPK activation(23). mTOR modulates global mRNA translation mainly through modulation of 4E-BPs and S6Ks(24, 25). However, mTOR also mediates selective mRNA translation (26). Upon activation, mTOR phosphorylates 4E-BPs leading to release of eIF4E(6, 14). As described above eIF4E then facilitates assembly of eIF4F, which is essential for cap dependent mRNA translation initiation. S6Ks (S6K1 and S6K2) phosphorylates multiple components of the translational machinery such as RPS6 which is implicated in ribosome biogenesis(27). Furthermore, S6Ks also phosphorylate eEF2 kinase which is a negative regulator of protein synthesis(28). Lastly, S6Ks phosphorylate programmed cell death 4 (PDCD4) triggering its SCF $\beta$ TrCP-dependent degradation (29). PDCD4 is a factor blocking the eIF4A-eIF4G interaction by binding to eIF4A. Binding of PDCD4 to eIF4A leads to inhibition of eIF4A activity and thus translation of mRNA that require RNA helicase activity(30). More recent work indicates an effect of mTORC1 on LA motif (LAM)-containing factor family



La-related protein 1 (LARP1). In that study the conserved RNA-binding protein of LARP1 interacts with raptor and is phosphorylated by mTORC1. However, the scope of LARP1 mediated effects remain controversial. It has been suggested LARP1 stabilizes or regulates translation of mRNAs with the terminal oligo pyrimidine (TOP) motif in a context dependent manner (18, 31, 32).

## 2. Aims of this thesis

The aims of this thesis are to explore the regulation of gene expression in cancer, more specifically we investigate perturbations of gene expression in different cancer models as a response to drug treatment.

In **Study I** we adapted an algorithm for ANalysis Of Translation Activity data (anota) so that it could be applied to next generation sequencing data. The resulting algorithm was named anota2seq.

We then applied the anota2seq algorithm to investigate changes in translation efficiencies in two cancer models:

In **Study II** we unravelled the effects of eIF4A, an RNA helicase, inhibition using a synthetic rocaglate CR-1-31-B (CR-31) in pancreatic ductal adenocarcinoma.

In **Study III** we explored the effects of insulin on gene expression in a breast cancer cell line.

### 3. Results and discussion

## 4. Conclusions

## Acknowledgments

Christina is awesome.

I am sorry for all the other people of this page but no one else helped me more than my 8 paws of awesomeness Felix and Dexter. These little litter shitters have been an extreme joy to be around and kept me sane during the insanity that is writing a thesis. **Meow**

## References

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