Contents

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

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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

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 inititiation is commonly regarded as the rate limiting step. Nevertheless, regulation

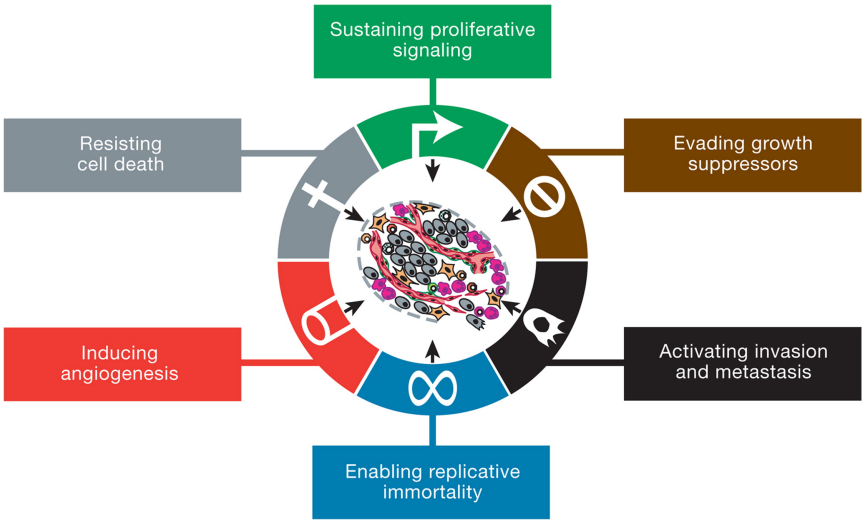

Figure 1.1: The Hallmarks of Cancer - This illustration encompasses the six hallmark capabilities originally proposed in our 2000 perspective. The past decade has witnessed remarkable progress toward understanding the mechanistic underpinnings of each hallmark. Reprinted from Hallmarks of cancer: The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

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-tRNAi) 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 facilitatedby 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. Afterrelease of eIF2-GTP the 60S ribosomal subunit joins to form the 80S ribosome and protein

synthesis can commence(Hinnebusch, 2014,Dever & Green (2012)). Next to this scanning mechanism, mRNA translation can also be initiated via alternative cap independent mechanisms(Wurth & Gebauer, 2015).

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-tRNAi 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 thatis 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(Dever & Green, 2012). 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(Dever & Green, 2012).

1.4. Regulation of mRNA translation

1.4.1. mTOR singalling 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 (deptor). mLST8 and deptor 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(**???**,Pearce et al. (2007)). 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-BPsvia phosphorylation of 4E-BPs by mTOR (Hsieh et al., 2010). Furthermore, substrates of mTORC1 include ribosomal S6 kinases (S6Ks) 1 and 2(Schepetilnikov et al., 2013), and La ribonucleoprotein domain family member 1 (LARP1)(Tcherkezian et al., 2014). mTORC2 is found to associate with ribosomes to promote co-translational phosphorylation and foldingofnascent Aktpolypeptide(Oh et al., 2010).As mentioned mTORC1 is activated via growth hormonesincludinginsulin and insulin like growth factor (IGF).For example,wheninsulin 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-biphoasphate (PIP2). This step is reversed by PTEN which hydrolyzes PIP3 to PIP2, thereby working antagonistically to PI3K. PIP3 recruits AKT and phosphoinositide-dependent kinase1 (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(Roux & Topisirovic, 2018,Roux & Topisirovic (2012),Laplante & Sabatini (2012)).Protein synthesis is the most energy expensive process within cells(Buttgereit & Brand, 1995).Therefore,regulation of mRNA translation is tied to cellular energy levels. AMP-activated protein kinase (AMPK) is a kinase activated by increased AMP/ATPratioas 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(Leibovitch & Topisirovic, 2018).mTOR modulates global mRNA translation mainly throughmodulation of 4E-BPs and S6Ks(Bruno D. Fonseca et al., 2014,Hay & Sonenberg (2004)). However,mTOR also mediates selectivemRNA translation (Gandin et al., 2016). Upon activation, mTORphosphorylates 4E-BPsleading to release of eIF4E(Roux & Topisirovic, 2018,Saxton & Sabatini (2017)). As described above eIF4E then facilitates assembly of eIF4F, whichis essential for cap dependent mRNA translation initiation. S6Ks (S6K1 and S6K2) phosphorylates multiple componentof the translational machinery such as RPS6which is implicated in ribosome biogenesis(B. Magnuson, Ekim, & Fingar, 2012). Furthermore, S6Ks also phosphorylateeEF2 kinase which is a negative regulator of protein synthesis(X. Wang et al., 2001).Lastly, S6Ks phosphorylate programmed cell death 4 (PDCD4)triggering its SCFbetaTrCP-dependent degradation (Carayol et al., 2008).PDCD4is a factor blocking the eIF4A-eIF4G interactionby binding to eIF4A.Binding of PDCD4 to eIF4A leads to inhibitionof eIF4A activity and thus translation of mRNAsthat require RNA helicase activity(Yang et al., 2003). More recent work indicates an effect of mTORC1on LA motif (LAM)-containing factor family La-related protein 1 (LARP1). In that study theconserved RNA-binding protein of LARP1interacts with raptor and is phosphorylated by mTORC1.However, the scope of LARP1 mediated effectsremaincontroversial.It has been suggested LARP1 stabilizes or regulates translation of mRNAs with the

terminal oligo pyrimidine (TOP) motif in a context dependent manner[Tcherkezian et al. (2014),Bruno D Fonseca et al. (2015),Deragon & Bousquet-Antonelli (2015)).

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 algortihm to invesitigate 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

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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**

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