Summary

Since the discovery of the RAS genes (HRAS, NRAS and KRAS), these were early on recognized as highly promising direct therapeutic targets and extensive research have over the last three decades brought insight into key and fundamental properties of RAS and revealed its involvement in tumorigenesis and several developmental disorders. Whereas aberrant regulation of RAS in somatic cells usually leads to cancer, germline mutations results in severe congenital syndromes such as Costello Syndrome (CS). The RAS genes encodes small monomeric GTPases that function as molecular switches to mediate downstream signaling from a variety of cellular receptors to promote cell proliferation and differentiation. Switching between the active (GTP-bound) and inactive (GDP-bound) states of the RAS proteins in response to cellular stimulation, is tightly regulated and binding as well as hydrolysis of GTP result in significant conformational changes in two functional regions termed switch I and switch II. A least one-third of all cancers harbor mutations in the RAS oncogenes of which the majority is located in exon 2. These mutations change codons 12 or 13 leading to production of a constitutively active RAS protein that prevent GTP hydrolysis, thus locking RAS in its active state. Despite the fact that the RAS GTPases are the most prevalent oncogenes in human cancer, previous attempts to therapeutically target RAS proteins has failed and it remains undruggable. The major focus of this thesis has been to understand the molecular mechanisms of RAS exon 2 splicing by focusing on a previously unrecognized Achilles heel of the RAS genes, namely exon skipping, to switch off constitutively active mutant RAS expression, which can be used as a new potential way to target oncogenic RAS. In Chapter 2 we demonstrate that mutations in codon 12 and 13 in HRAS differently affect splicing efficiency and thereby determined HRAS protein activity. We investigate the role of a c.35_36GC>TG dinucleotide variation (substitution of glycine 12 to valine 12) in a patient with an attenuated CS phenotype that abolishes an SRSF2-binding exonic splicing enhancer (ESE) and generates an exonic splicing silencer (ESS), which binds hnRNPF/H. Shifting the balance of these splicing regulatory elements causes pronounced skipping of exon 2 and abrogate formation of the constitutively active HRAS protein, which could explain the mild clinical phenotype. We demonstrate that HRAS exon 2 is a vulnerable exon holding a weak 3' splice site and polypyrimidine tract (PPT), which makes it a suitable target for splice switching oligonucleotide (SSO) based therapy to induce exon skipping and halt cancerous cell proliferation

In Chapter 3 we find that the PPT of both HRAS and NRAS is interrupted by five purines, but, different from NRAS, the PPT of HRAS holds a GGG triplet as well as a flanking GGG triplet downstream from position +3 of the exon. These triplet GGGs functions as silencers, which bind hnRNPH/F, causing HRAS to possess a functionally weaker 3' splice site than NRAS. We designed SSOs to cover the entire exon 2 of HRAS and employed HRAS minigenes with serial deletions to delimitate new potential splicing regulatory elements (SREs) to target. Using this approach, we identified a new downstream enhancer element region. Based on the localization of this essential ESE, we successfully demonstrate that SSO-mediated exon 2 skipping in T24 bladder cancer cells disrupt HRAS protein function and decreases cell proliferation. We show that that the efficiency of SSOs can be increased upon addition of a non-hybridizing tail sequence carrying hnRNPF/H-binding GGG triplets to enhance exon silencing.

Interestingly, we find that a T-to-C transition SNP at position c.81 of HRAS exon 2 is covered by our targeting SSO. Using tailed length-reduced SSOs harboring locked nucleic acid (LNA) modifications we selectively target the c.81T>C SNP, which could provide an allele specific therapeutic strategy for Costello patients heterozygous for this SNP and as an anti-cancer strategy.

In Chapter 4 we employed these key findings to oncogenic KRAS that is mutated in three of the deadliest cancers namely lung, colorectal and pancreatic cancer. We identify a potential SSO, which induces exon 2 skipping and abolish KRAS protein expression facilitating downregulation of cell proliferation in lung and colorectal cancer cells. In Chapter 5 we investigate and discuss the in vivo role of KRAS SSO-based therapeutics using lung and colorectal xenograft mouse models.