SUMMARY

Cancer is the result of a multistep process involving the gradual change of healthy cells into immortal and often invasive cells. This process is called oncogenic transformation and requires the evasion of cell-intrinsic failsafe mechanisms that normally limit uncontrolled proliferation. Induction of a permanent cells cycle arrest in response to oncogenic stress, a process termed oncogene-induced senescence (OIS), represents such a built-in tumor-suppressive mechanism. Indeed, work of recent years clearly shows that OIS acts as a powerful pathophysiologic mechanism suppressing cancer, both in animal models and in humans. Also, it has become clear that factors controlling OIS are endowed with tumor-suppressive functions and are often absent or mutated in cancer. In this thesis, we have studied genes and signaling pathways critical for OIS in order to find factors important for oncogenic transformation. We have also made an attempt, wherever possible, to translate our findings to a clinically relevant setting.

In **Chapter 1,** we introduce major cellular metabolic pathways and give an overview of metabolic changes occurring in malignant cells. Besides that, we review the current knowledge on metabolic regulation in senescence. To date, only few studies on that subject have been reported, showing that senescent cells rewire their metabolism in a way that counteracts changes seen in the cancer cells. Hence, the antitumor function of senescence is manifested also on the level of metabolic regulation.

In **Chapter 2,** we give an account of how proliferation pathways, the cell cycle machinery and metabolism are interconnected. We illustrate that the molecular mechanisms underlying metabolic reprogramming involve not only alterations in multiple proliferation pathways, with PI3K/AKT/mTOR signaling as a prime example, but also direct communication between the components of the cell cycle machinery and metabolic enzymes.

Chapter 3 presents our analysis of metabolic changes in OIS. Through mass balance analysis and metabolic flux profiling we show that OIS cells have a distinct metabolic profile when compared to cycling cells. Specifically, entry into OIS is associated with an increased rate of pyruvate oxidation in mitochondria, lower utilization of glutamine and a higher rate of fatty acid secretion. Notably, these changes in metabolism oppose those in cancer.

In **Chapter 4,** we demonstrate that increased glucose oxidation in mitochondria in OIS is regulated by the mitochondrial gatekeeper pyruvate dehydrogenase (PDH). In OIS, PDH is activated upon downregulation of its inhibitory kinase PDK1 and simultaneous upregulation of PDH-activating phosphatase PDP2. Importantly, the abrogation of OIS, a rate-limiting step towards oncogenic transformation, coincides with reversion of these processes. This shows that PDH (de)regulation is a direct mediator of, rather than a phenomenon only associated with OIS. Further supporting a critical role of PDH in OIS, enforced normalization of either PDK1 or PDP2 expression levels inhibits PDH and abrogates OIS, thereby licensing melanoma development. Finally, depletion of PDK1 causes regression of established BRAF-driven

melanoma and eradicates melanoma subpopulations resistant to targeted BRAF inhibition. These results reveal a mechanistic relationship between OIS and a key metabolic signaling axis, which may be exploited therapeutically.

Chapter 5, **Chapter 6** and **Chapter 7** describe unbiased screening approaches aimed at the identification of novel OIS regulators with a potential tumor suppressive function. In **Chapter 5** we have used a function-based short hairpin (sh)RNA screen and identified seven genes, depletion of which abrogates senescence-associated cell cycle arrest. By genome-wide promoter methylation analysis we found that one of the genes, *RASEF*, is hypermethylated in melanomas when compared to senescent nevi. While RASEF depletion abrogates OIS, causing continued cell proliferation, restoration of its expression in melanoma acts cytostatically. This is in agreement with a potential tumor suppressive role of *RASEF.* In **Chapter 6** and **Chapter 7,** we describe our mass spectrometry-based analysis of the proteome and phosphoproteome in cycling, senescent, and senescence-escaping cells. Among senescence-regulated proteins, we found several previously established senescence mediators, including cell cycle regulators and cytokines, but also multiple proteins previously not associated with the senescence program.

In **Chapter 8,** we discuss our findings in more detail and speculate on their potential future implications. In addition to that, we formulate remaining and newly arisen questions concerning the mechanism controlling OIS and oncogenic transformation that need to be answered to better understand the complexity underlying tumorigenesis and to uncover new targets for therapeutic intervention.