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Toward a non-coding RNA revolution in cancer society

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MicroRNAs were linked to the progression of all types of human tumors that were investigated to date. The main molecular alterations are represented by variations in gene expression, usually mild and with consequences for a vast number of target protein coding genes. Recent studies proved that miRNAs are main candidates for the elusive class of cancer predisposing genes and that other types of non-codingRNAs participate in the genetic puzzle giving rise to the malignant phenotype. These discoveries could be exploited for the development of useful markers for diagnosis and prognosis, as well as for the development of new RNA-based cancer therapies.

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MicroRNA-221/-222 pathway controls melanoma progression

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The incidence of cutaneous melanoma is steadily increasing. Although several molecular abnormalities have been associated with melanoma progression, the mechanisms underlying the differential gene expression are still largely unknown and targeted therapies not yet available.

Non-coding small RNAs, termed microRNAs (miRs), have been recently reported to play important roles in the major cellular processes, including those involved in cancer development and progression. We have identified the promyelocytic leukemia zinc finger (PLZF) transcription factor, as a repressor of miR-221 and miR-222 by direct binding to their putative regulatory region. Specifically, PLZF silencing in melanomas unblocks miR-221 and -222, which in turn controls the progression of the neoplasia through downmodulation of p27Kip1/CDKN1B and c-KIT receptor, leading respectively to enhanced proliferation and differentiation blockade of the melanoma cells.

In vitro and in vivo functional studies, including the use of antisense “antagomir” oligonucleotides, confirmed the key role of miR-221/-222 in regulating the progression of human melanoma suggesting that targeted therapies against miR-221/-222 may prove beneficial in advanced melanoma.

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SYMPOSIUM

Ageing telomerase

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Oncosuppressive effects of telosome protein inhibition

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This study was designed to investigate the effects of inhibition of telosome proteins on tumorigenicity. We observed that the expression of a dominant negative form of TRF2 or the inhibition of POT1 and TRF2 expression by RNA interference markedly reduced the ability of transformed human fibroblasts to form tumour in immunosuppressed mice. Strikingly, in the absence of overt telomere damage, growth defect, viability, apoptosis or senescence, TRF2-compromised cells exert a paracrine effect on tumor growth. These results uncover that TRF2 inhibition alters the microenvironment of transformed cells in such a way that tumour formation is impaired. They imply that the role of telomeres in oncogenesis can be uncoupled from a direct effect on the proliferation of telomere-compromised cells. They also enlighten telosome inhibition as an effective therapeutic strategy, even in the setting of p53- and Rb- compromised tumours.

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Telomere length and haematopoietic stem cell adaptation in mice compromised for telomerase activity

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Heterozygous mutations of the human telomerase reverse transcriptase (Tert) and the telomerase RNA (Terc) are linked to autosomal dominant dyskeratosis congenita (AD-DKC), aplastic anemia, and pulmonary fibrosis. In mice, mTerc heterozygosity also leads to telomere erosion and phenotypes associated with loss of telomere function, including defects in bone marrow and reduced lifespan [1]. We examine the impact of sustained heterozygosity of the murine telomerase reverse transcriptase mTert and, in particular, the impact upon haematopoietic stem cells (HSC). We published previously that successively interbred mTert nullizygous animals, like mTerc-/- animals, display a defect in HSC function and fertility [2]. Although a comparable telomere erosion also occurred in successively maintained mTert+/- animals, a minimal telomere signal was maintained at all chromosome ends, and no phenotypic defects were observed [2]. Thus, we continued to follow successive mTert+/- generations (by breeding to wild-type or mTert+/- animals) to examine the eventual consequences upon HSC function. Surprisingly, beyond 10 mTert+/- generations, telomere lengths were stabilised (and in some cases, elongated) relative to previous generations. As a consequence of this telomere adaptation, the first generation mTert null progeny generated from mTert+/- interbreeding possessed telomere lengths comparable to wild-type or mTert+/- littermates. Consistent with the lack of ongoing telomere erosion, no mTert+/- generation exhibited any observable phenotypic defects. Nullizygous progeny of mTert+/- parents, maintained in an mTert null background for three generations, exhibited significant defects in fertility and intestinal cell viability, but showed no defects in HSC function, even after challenge with radio-reconstitution and serial HSC transplantation. Our data establish that in vivo telomere length adaptation occurs upon prolonged exposure to limiting telomerase levels, leading to a preservation of HSC function even in subsequent progeny that lack telomerase altogether.

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Role of Exo1 in telomere biology

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Telomeres shorten which each round of cell devision due to the end replication problem of DNA polymerase and due to processing of telomeres during the cell cycle. When telomeres reach a critically short length they lose capping function at the chromosome end. Dysfunctional telomeres induce DNA damage responses limiting the lifespan of primary human cells by induction of senescence or apoptosis. We have recently provided experimental evidence that telomere shortening limits the function of adult stem cells by activation of cell intrinsic checkpoints and by induction of environmental alterations. Moreover, our studies provide first experimental evidence that the deletion of DNA damage checkpoints can improve stem cell function, organ maintenance and lifespan of telomere dysfunctional mice. Deletion of Exonuclease-1 (Exo1) rescues both the induction of apoptosis and cell cycle arrest in telomere dysfunctional mice. Our studies show that Exo1 acts upstream of p53 inhibiting the formation of single stranded DNA and activation of ATR in response to irradiation and telomere dysfunction. Of note, deletion of Exo1 does not increase chromosomal instability and cancer formation in aging telomere dysfunctional mice. These data indicate that Exo1 represents a promising target for future therapies aiming to improve regenerative capacity in disease stages associated with telomere shortening and regenerative exhaustion, such as liver cirrhosis.