to an increased risk of cancer. Somatic mutations have also been found in sporadic lung adenocarcinomas.

Sequencing of 154 lung cancer cell lines has revealed an apparent clustering of LKB1 mutations with KRAS or BRAF mutations in non small cell lung cancers (p-value 2.34x10-5). The two signaling pathways containing LKB1 and KRAS or BRAF are linked via Rheb, a GTPase and member of the Ras superfamily. Overexpression of Rheb leads to overactivation of mTOR and inhibition of wild type BRAF and therefore the Ras-MAPK signaling pathway. Thus, Ras-MAPK pathway mutations may be necessary in a subset of LKB1 null lung cancers to maintain proliferative advantage.

We have undertaken experiments to test this hypothesis in LBK1/Ras-MAPK mutant lung cancers using MEK inhibition as a targeted approach. The data shows that cell lines with LKB1 and KRAS mutations are more sensitive to MEK inhibition, than KRAS mutant cell lines or cell lines wild type for both genes. Western blot analysis of phosphorylated ERK confirms the inhibition of MEK and we are now undertaking siRNA knockdown of components in both pathways.

Gene expression analysis of LBK1/Ras-MAPK mutant lung cancers has revealed alteration of a large number of metabolic pathways, which would further confirm the role of LKB1 in regulating response to energetic stress. This may also provide a possible mechanism by which these LBK1/Ras-MAPK mutant lung cancers avoid the premature senescence observed in LKB1 null MEFs when transformed with oncogenic RAS. From this analysis there are a number of genes we are following up with western blot analysis, siRNA knockdown and immunocytochemistry.

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Control of ribosome biogenesis by oncogenic ETS transcription factors

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The ETS family comprises about 30 transcription factors sharing a consensus DNA binding sequence and several of them are oncogenic. For example, FLI-1 which is normally expressed in megakaryocytes and macrophages but downregulated in erythroid cells, is recurrently involved in erythroleukemia. Indeed, the fli-1 gene is constitutively activated in most of clonal erythroleukemia induced by the Friend virus in mouse. Friend's erythroleukemia cells proliferate without growth factors and are blocked at an early stage of differentiation. We showed using shRNA mediated inducible fli-1 knock-down that FLI-1 indeed contributes to the proliferation, survival and differentiation block of Friend's erythroleukemia cells. We then search for new FLI-1 target genes potentially involved in its oncogenic properties by a transcriptomic approach. Among genes activated by FLI-1, we identified a surprisingly high proportion of genes involved in ribosome biogenesis in the nucleolus. Real-time RT-PCR confirmed that the expression level of these genes decreased following fli-1 knockdown and in vivo chromatin immunoprecipitation (ChIP) experiments revealed FLI-1 occupancy at their promoters thus indicating that they are direct FLI-1 target genes. Interestingly, increased ribosome biogenesis resulting in an hypertrophied nucleolus is a well known feature of cancer cells. Based on these results we hypothesized that the control of ribosome biogenesis by ETS factors could be a general mechanism potentially used by oncogenic ETS factors to modify the ribosomes quality or quantity in cancer cells. In agreement with this hypothesis, we found that the promoters of these genes are also bound by others oncogenic ETS factors such as SPI-1/PU.1 in Friend erythroleukemia cells and ETS1 and ETS2 in human prostate cancer cell lines. Two alternative, although not mutually exclusive, mechanisms can be considered to explain how FLI-1 and others oncogenic ETS factors could favor cell transformation by impacting on ribosome biogenesis. The first possibility could be that oncogenic ETS factors modify the ribosome quality, resulting in altered translation of some specific mRNA encoding proteins which themselves contribute to cell transformation. Alternatively, oncogenic ETS factors could favor ribosomes production and by this way contribute to the increased growth rate of cancer cells. We are now using FLI-1 in erythroleukemic cells as a model to distinguish between these two possibilities.

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A conserved mechanism to maintain the spindle checkpoint: Cdc20 switches from an APC/C activator to a substrate

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The proper control of mitosis depends on ubiquitin-mediated proteolysis of key substrates at the correct time. Crucially, securin and Cyclin B1 must not be degraded until all chromosomes are properly attached to the spindle. The spindle assembly checkpoint (SAC) is activated by unattached/improperly attached kinetochores, and once activated it prevents the anaphase Promoting Complex/Cyclosome (APC/C) from ubiquitinating Cyclin B1 and securin. It has been shown the primary target of the SAC is Cdc20/Fizzy, the APC/C activator. However, It is unclear how the SAC prevents Cdc20 from activating APC/C to degrade Cyclin B and Securin.To gain a better understanding of how the SAC regulates Cdc20 we combined biochemical analysis with a live cell assay monitoring the behaviour of YFP tagged Cdc20 by time-lapse fluorescence and DIC microscopy. Although Cdc20 levels did not normally change during a checkpoint arrest, Cdc20 accumulated in nocodazzole arrested when we added proteasome inhibitor. Cyclohexamide chase studies in Hela cells showed that the half-life of cdc20 in checkpoint arrested cells was 30min. Consistently, in live cell assay, YFP-Cdc20 levels began to decline as soon as we added nocodazole to cells in prometaphase or metaphase. Depletion of APC3 stabilized Cdc20 in the experiments above, suggesting that APC/C is the ubiquitin ligase responsible for targeting Cdc20 to degradation.We analyzed nocodazole or taxol blocked mitotic cells by gel filtration column chromatography to identify in which complex Cdc20 accumulated when we prevented the turn-over. We blocked the proteasome with MG132. This showed that Cdc20 accumulates in the complex containing BubR1 and APC/C. These results were consistent with Cdc20 binding to the BubR1 complex when the checkpoint was active to be presented to the APC/C as a substrate for ubiquitination. To determine the importance of ubiquitination and degradation of Cdc20 to the SAC we made a mutant of Cdc20 that could not be ubiquitinated by changing all he lysines to arginines. This K23R mutant of Cdc20 was functional because it could rescue the mitotic arrest induced by siRNA directed against Cdc20 with a similar timing to wild type Cdc20. Moreover, It was able to drive checkpoint-arrested Hela and RPE cells out of mitotsis whereas wild type Cdc20 could not. Microinjecting cells with various levels of the K23R mutant demonstrated that even low levels were sufficient to override the checkpoint. K23R mutant is still able to bind to Mad2 and form complexes with BubR1. Therefore, our data indicates that Cdc20 ubiquitination is required to maintain the SAC. Moreover, since Cdc20 that cannot be ubiquitinated is able to replace wild type Cdc20 to promote mitotic exit, and can do so even in the presence of nocodazole or taxol, we find no evidence that cdc20 ubiquitination is important to inactivate the checkpoint as it had been suggested in two recent studies.

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Hif-2alpha mediates UV induced apoptosis through a novel ATF3 dependent death pathway

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Environmental genotoxic stress such as UV light is one of the major causes of genomic instability. During such a stress, normal cells orientate their physiological response toward DNA repair or, if the extent of damage is too severe, undergo apoptosis. This process, deleterious for cell survival, is crucial because it leads to the destruction of cells that bear the risk of becoming tumorigenic. Such an anti-tumor mechanism involves the regulation of specific genes under the control of signaling pathways initiated by key transcription factors.

In this particular context, we have identified a novel ATF3 dependent death pathway triggered by UV irradiation. We have demonstrated that ATF3 transcription factor contributes to UV induced apoptosis, through the regulation of Hif-2alpha expression which in turn induces expression of pro-apoptotic genes, such as Casp7 or TRAIL. Gain of funtion of Hif-2alpha as well as ATF3 is sufficient to trigger cell death, while loss of function of both proteins drastically inhibits UV induced apoptosis. Repression of Hif-2alpha, by a siRNA approach, strongly impairs ATF3 mediated death, providing evidences that Hif-2alpha is the major death effector of ATF3. In addition, Hif-1alpha, already known as pro-apoptotic gene upon UV irradiation, is not able to compense lack of Hif-2alpha expression, confirming thus the major contribution of Hif-2alpha in UV mediated cell death. We further demonstrate that this cascade of gene activation depends on p38 and JNK activity. Impairment of such a pathway is likely to contribute to oncogenesis by promoting survival of cells that could accumulate severe chromosomal alterations.