

from culture to epithelial-like cells expressing EGFR in tumours indicates deregulation of cell surface molecules possibly induced by epigenetic stress such as hypoxia and local factors in a different environment. This study was conducted abiding by the regulations of the Ethic Committee of the Hebrew University – Hadassah Medical School, Jerusalem, Israel.

[390] Enhanced suppression of Raf-1 activity by K vitamins mediates their synergy with Sorafenib to inhibit HCC cell growth in vitro and in vivo

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Sorafenib is a multi-kinase inhibitor that has been approved for treatment of human hepatocellular carcinoma (HCC). However, Sorafenib-induced tumour shrinkage is minor. Naturally occurring K vitamins are blood coagulation cofactors, which have also been reported to possess weak inhibitory effects on hepatoma cell growth. To enhance Sorafenib inhibition of HCC growth, we developed a new strategy to combine K vitamins with Sorafenib, and found that K vitamins synergized with Sorafenib to inhibit HCC cell growth in culture and in a rat transplantable HCC model. To explore the mechanisms involved, we examined the role of Raf-1 kinase, because it has been reported that Sorafenib inhibits tumour cell growth as a direct inhibitor of Raf, whereas vitamins K can induce PKA activity, which is a Raf-1 upstream kinase. We found that while lower doses of vitamin K1 or K2 (10 mM) or Sorafenib (2.5 mM) alone slightly induced Raf-1 phosphorylation at both Ser-43 and Ser-259 in Hep3B cells, combination of vitamin K1 or K2 plus Sorafenib treatment resulted in strong Raf-1 phosphorylation at these two Raf-inhibitory serine residues. Raf-1 protein kinase activity assay showed that lower concentrations of vitamin K1 or K2 or Sorafenib alone had little, if any effects on Raf-1 activity, whereas combination of vitamin K1 or K2 plus Sorafenib at the same low concentrations significantly inhibited Raf-1 activity, judged by strong inhibition of MEK, and of ERK phosphorylation, the Raf downstream targets. Since Raf-1 phosphorylation at Ser-43 and Ser-259 can be regulated by either PKA or Akt kinase, we examined the effects of both vitamin K1 or K2 or Sorafenib on their phosphorylation. Although both vitamin K1 or K2 or Sorafenib alone induced PKA phosphorylation, no synergistic phosphorylation effects on PKA were found using the combination. However, vitamin K1 or K2 enhanced Sorafenib-induced Akt phosphorylation, which was associated with enhanced c-Met tyrosine phosphorylation at Tyr-1349, a known phospho-Akt regulator. Our data suggest that enhanced inhibitory Raf-1 phosphorylation at Ser-43 and Ser-259 may play an important role in K vitamin plus Sorafenib synergy in inhibiting HCC cell growth.

[391] Heterogeneous tumorigenic ability of human brain tumour derived neurospheres

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Background: Gliomas constitute an highly heterogeneous group of tumours. To date, glioblastoma (GBM) remains one of the most difficult cancer to treat. Several evidences emerged in the last years suggest that tumours growth initiate and is sustained by a subpopulation of cells called cancer stem cells (CSCs). Recent findings affirm that tumours consist of heterogeneous populations of cells differing in markers expression and growth capacities.

Materials and Methods: Tumours sample were dissociated and the cells obtained cultured in proliferative or differentiating medium. CD133 cells were selected by immunomagnetic separation and clones obtained by limiting dilution assay. Cytogenetic was performed on metaphases chromosome obtained by adding colchicine to the medium. Cells were incubated in hypotonic solution, fixed and chromosomes were Q-banded. Chicken chorio-allantoic membrane (CAM) experiments: GBM growth was obtained grafting the cells on the membrane surface. 7 days after implantation, CAMs were harvested and frozen for immunofluorescence analysis. Intracranial experiments: nude mice received stereotactical injection of different groups of cells into the left forebrain; at different time point animals were sacrificed and the brain frozen for subsequent analysis.

Results: Tumour-derived spheres express high levels of nestin and differentiate in the 3 neural lineages. Cytogenetic analyses revealed a near tetraploid karyotype with an average of 75–90 chromosomes. After intracranial transplantation in nude mice in different sets of experiments, in toto neurospheres derived from one GBM and CD133+/CD133– cells showed different degrees of brain modifications. To understand this biological heterogeneity we derived single cell clones from the tumour derived neurospheres. Among these clones we noticed different growth rates even

if they retain the same ability to differentiate. We then implanted the clones in CAM model and after in nude mice. The results confirmed the different growth rate among the clones in both the animal models.

Conclusion: These findings demonstrated the existence of different cell types within tumour spheres confirmed by the presence of different proliferation rates that characterize every single cell. These data reveal that these heterogeneity matches exactly the CSCs hypothesis, that describe the tumours as hierarchical organization of malignant cells, with the clonal evolution, which determines uncontrolled proliferation and differentiation of tumour cells.

[392] Isolation and characterisation of lymphoma stem cells

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Cancer stem cells (CSCs) or tumour-initiating cells (TICs) have been described for a number of malignancies and are deemed responsible for continued growth and propagation as well as for disease relapse and in some cases metastatic growth of many cancers. It has yet to be determined whether CSCs/TICs exist for Non-Hodgkin lymphoma (NHL), a heterogeneous disease affecting the cells of the immune system. There are greater than 30 sub-types of NHL and our initial focus is on the Peripheral T-cell Lymphoma-Not Specified Otherwise (PTCL-NOS) and Anaplastic Large Cell Lymphoma (ALCL) categories, both representing mature T-cell malignancies. In this study, we provide evidence for the presence of a distinct sub-population of cells with stem-like properties within the bulk tumour population. We have employed the Side Population (SP) technique to identify cells with enhanced drug efflux capacity, an inherent property of stem cells and hence believed to be enriched in the SP population. We show that a distinct population of SP cells exist in ALCL cell lines and that this population contains cells positive for the Haemopoietic Stem Cell (HSC) surface markers CD117 (2–5%), CD90 (8–10%) and CD34 (1–3%) which are absent in the main population (MP). Furthermore, *in-vitro* differentiation assays show that the isolated SP population proliferates at a significantly greater rate than the MP cells. We have also identified a verapamil-sensitive SP population in primary human ALCL and PTCL-NOS patient samples and furthermore we demonstrate that 2–3% of these cells express CD117 on their surface. These data suggest that there may be a population of CSCs/TICs within NHL populations. It remains to be determined whether these cells whilst resembling tissue-specific stem cells function in a similar manner, i.e. differentiate to give rise to the bulk tumour population. However, limiting dilutions of primary patient biopsy specimens on engraftment into immunodeficient mice produce tumours with a latency reflective of the lymphoma cell dilution. The tumours that develop also contain a small population of CD117-expressing cells. Hence, we propose that at least some NHL may constitute a small population of CSCs/TICs.

[393] NPAS3 is a novel late-stage acting progression factor in gliomas with tumour suppressive functions

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Background: In our effort to better comprehend the genetics of gliomas, we explored new therapeutic targets. We previously cloned NPAS3, a transcription factor which maps to human chromosome 14. Our principal aim is to comprehend the disease associations of NPAS3, since we recently identified expression in human astrocytes. We investigated NPAS3 as a candidate for astrocytomas based on findings archived from the Cancer Genome Project demonstrating a loss of NPAS3 expression and with loss-of-function deletions of human chromosome 14 with NPAS3 in 30–50% of astrocytomas.

Methods and Results: After undertaking extensive functional analyses, we now have novel evidence supporting NPAS3 as an astrocytoma tumour suppressor involved in late-stage tumour progression, based on: (1) Absent NPAS3 expression is predominant in high grade astrocytomas (79–83%), in comparison to low grade astrocytomas (29–35%). (2) Loss of function mutations of NPAS3, which are associated with a loss of heterozygosity of the NPAS3 locus are identified in GBMs. (3) Absent NPAS3 expression is predominant in >60% of malignant human glioma cell lines. (4) An over-expressed NPAS3 in malignant glioma cell lines suppresses the transformation potential, while the converse reduced expression promotes an increase in transformation potential. (5) A reduced NPAS3 expression (efficiency >90%) in concert with other gliomagenesis genes can transform a well characterized TERT immortalized human astrocyte cell line and promote the growth of anaplastic astrocytomas.

Conclusions: Our data provide compelling findings of NPAS3 as a novel gene involved in the cause of astrocytomas, with tumour suppressive and late-stage acting progression factor roles.