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Molecular modelling of KIT and PDGRA mutant forms to predict sensitivity to new drugs in GISTs

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Background: Gastrointestinal stromal tumours (GISTs) are the most frequent mesenchymal tumours of the gastrointestinal tract. The molecular event determining GIST development is the activation of the KIT receptor or, alternatively, PDGFRA (hereafter RTKs), and their pathways. Imatinib-mesylate (Gleevec®, Novartis Pharmaceuticals) has been demonstrated to be able to block the activation of both the receptors and to inhibit GIST proliferation.
Evidence derived from clinical experience points out that GISTs respond well to this therapy and that different RTK mutation types correlate with a different response rate to Imatinib. However, an acquired resistance is often associated with the presence of secondary mutations affecting the ATP pocket of those RTKs. It is therefore necessary to develop second line therapies that could be greatly improved by a clearer understanding of the inhibition mechanisms eventually leading to the identification of new therapeutic strategies.
Material and Methods: Tumour specimens from different patients were analysed biochemically to determine KIT/PDGFRA activation and sequenced for the corresponding genes. Subsequently, an "in-silico" approach was developed by reproducing the 3D structure of the different RTK forms, while the affinity for ATP and the analysed drugs were calculated using the theoretical framework of the Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) method. Finally "in-vitro" cell lines expressing the different RTK forms were biochemically analysed to determine the RTK activation status.
Results: A good correlation between the "in silico" results and the biochemical analyses of the "in vitro" cell lines was observed in different instances and has also been observed "in vivo" in the treated patients.
Conclusions: This integrated multidisciplinary approach demonstrates that computer-based molecular simulation can be used as a reliable technique to estimate the affinity of binding between receptors and inhibitors. Accordingly, "in silico" approaches together with "in vitro" genetically engineered cell lines could be employed to predict the biological response to small molecules inhibiting RTKs. An analogous approach could then be utilized to screen new potentially therapeutic drugs in a rapid and efficient way.

114 EACR Young Cancer Researcher Award

Chromatin modulates DNA damage response activation in oncogene-expressing cells

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Early tumorigenesis is associated with the engagement of the DNA-damage checkpoint response (DDR). Cell proliferation and transformation induced by oncogene activation are restrained by cellular senescence. We have previously shown that retroviral-mediated expression of an activated oncogene in cultured normal human cells results in a permanent cell-cycle arrest caused by the activation of a robust DDR. Experimental inactivation of DDR abrogates senescence and promotes cell transformation. Oncogene-induced senescence is also associated with a global heterochromatinization of nuclear DNA. Senescence-associated heterochromatic foci (SAHFs) are enriched in heterochromatin markers and they have been proposed to enforce cellular senescence by suppressing the expression of proliferative genes.
Presently, DDR activation and heterochromatinization are considered the two main tumor suppressors mechanisms that control cellular senescence. However, whether these are independent pathways or whether there is an interplay and they modulate each other is unknown.
We have discovered that chromatin changes induced by oncogenes occur in a DNA replication-dependent manner and, differently from what had been previously proposed, that heterochromatin formation is not involved in the suppression of proliferative genes. Instead, our results indicate that cells expressing an oncogene induce chromatin changes to constrain DDR spreading and reduce signaling. Indeed, we have observed that heterochromatin reduction reproducibly leads to augmented DDR signaling and altered cell viability of oncogene-expressing senescent cells.
Our observations have potential important implications for the therapeutic treatment of cancers.

Keynote Lecture (Tue, 22 Sep, 11:15−12:00)
Targeting the cell cycle in cancer

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Targeting the cell cycle in cancer

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Unicellular organisms such as yeasts require a single cyclin-dependent kinase, Cdk1, to drive cell division. In contrast, mammalian cells are thought to require sequential activation of at least four different Cdks, Cdk2, Cdk3, Cdk4 and Cdk6, to drive cells through the different phases of the cell cycle interphase (G1, S and G2), as well as Cdk1 to proceed through mitosis (M phase). This model has been recently challenged by genetic evidence illustrating that mice survive in the absence of individual interphase Cdks and that most cell types proliferate in the absence of multiple interphase Cdks (Malumbres and Barbacid, Nat Rev Cancer, 9, 153, 2009). Indeed, we have recently shown that mouse embryos lacking all interphase Cdks (Cdk2, Cdk3, Cdk4 and Cdk6) undergo organogenesis and develop to midgestation, indicating that Cdk1 alone can efficiently drive the cell cycle even during one of the most active periods of cell proliferation (Santamaría et al., Nature 448, 811, 2007). Similar results have been recently obtained in adult mice. On the other hand, interphase Cdks are essential for proliferation of highly specialized cell types such as pancreatic beta cells, embryonic cardiomyocytes or hematopoietic precursors. Thus, suggesting that the generation of multiple interphase Cdks during eukaryotic evolution was required to drive the cell cycle in specialized cell types rather than to contribute to progression through the basic phases of the mammalian cell cycle.
Based on these observations, we have reasoned that individual Cdks might also be selectively required to drive the cell cycle of certain tumor cell types, depending on their tissue/cell type of origin and/or the driving oncogenic mutations. To test this hypothesis, we have used a well-characterized animal tumor model for non small cell lung carcinoma (NSCLC) driven by an endogenous K-RasG12V oncogene that can be activated at will in postnatal mice. In these mice, we have systematically ablated each of the interphase Cdks either in the germ line or in adult mice following a conditional strategy after tumors could be observed by CT analysis. Neither ablation of Cdk2 or Cdk6 had significant effects in the reduction of tumor burden or in survival. In contrast, ablation of Cdk4 resulted in a drastic reduction of tumor number as well as tumor size and grade. Mechanistic studies have revealed that lung alveolar cells selectively require Cdk4 to avoid entering senescence when they express a resident K-RasG12V oncogene. No induction of senescence could be observed in other cell types or upon ablation of other interphase Cdks such as Cdk2. These observations indicate that tumor cells also have specific requirements for selective interphase Cdks to drive their own cell cycle. Comparison of these results with pharmacological intervention using selective Cdk4 inhibitors should help us to establish a therapeutic strategic that may be eventually applicable to cancer patients.

Special Session (Tue, 22 Sep, 13:30−14:30)
National cancer plans: one size doesn't fit all

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The Netherlands cancer plan experience

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The experience in the Netherlands with national cancer plans has been rather mixed, so that lessons can be drawn: plans were/are only useful if they address the demographic and technological 'future' (adapting is already a great virtue) and need to be in line with 'the' Dutch Cancer Society (dominating the Dutch cancer control scene since the mid 50's), raising money for change (i.e. research and awareness), but also raising enough interest for (and satisfying!) the various – often autonomous – planning and programming agencies (around the Hague centre of polical power) and the more regionally oriented health insurance companies (going from 40 to <10) that fund the – now 8- Comprehensive Cancer Centres with their quality of care promoting role based on hosting the regional cancer registries. They are confronted with or driven by the aspirations of the various professionals (by definition renewing themselves in international perspective) and increasingly by the patients, who now command a strong national organisation, albeit being as diverse as there are cancers and becoming more voal with rising prevalence. Their motives are largely determined by their spokespersons who, besides willing to live, are mostly interested in quality of life and want to taken seriously.