Johnson Matthey PLC is also acknowledged for their generous gift of K_2PtCl_4.

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A broad panel of screening assays for mutation and methylation assessment of genes involved in the pathological development and therapeutic treatment of NSCLCa broad panel of screening assays for mutation and methylation assessment of genes involved in the pathological development and therapeutic treatment of NSCLC: utility for therapeutic monitoring

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Non-small cell lung cancers (NSCLC) contain numerous alterations, including somatic mutations and CpG island hypermethylation in a variety of genes that contribute to the pathological phenotype. Some of these tumor-associated variations have also been detected in the DNA found in the plasma of NSCLC patients, including DNA with mutations in TP53 and KRAS, and hypermethylation in p16INK4a and MGMT. However, a more comprehensive study designed to assess both mutation and methylation status of NCSLC patients at various stages has not been reported. Here we describe a combined assay panel for the assessment of genes associated with disease pathogenesis and therapeutic treatment of NSCLC. DNA from primary tumors and matching patient plasma were screened for mutations in a variety of genes including PTEN, TP53, TP73, KRAS, NRAS, BRAF, MET, EGFR, PDGFRA, PDGFRB, and KIT, and alterations of methylation status in APC, MLH1, p16, p14, MGMT, GSTP1, DAPK, RASSF1A, RUNX3, RARB2, and FHIT. We chose mutation and methylation scanning technology and these targets to develop a thorough screening methodology for alterations known to be associated with NSCLC or that may be involved in resistance to targeted therapeutics. Variations were identified with a denaturing high-performance liquid chromatography (DHPLC) platform that uses post-separation fluorescence technology, enabling the detection of variants that represent <0.1–1.0% of the total analyzed DNA. Using this approach, we identified at least one somatic or epigenetic event in 100% of the NSCLC patients. In no case was a mutation found in the primary tumor that was not also present in the plasma. The results emphasis the heterogenous pattern of genomic alterations and that scanning provides an attractive approach to comprehensive NSCLC genetic and epigenetic screening. The thoroughness of approach may have important implications for screening and staging, and disease monitoring during and following therapy.

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Metabolic activation of satraplatin by haemoglobin in vitro

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Background: Satraplatin is an orally administered Pt(IV) drug that has shown clinical activity against advanced prostate and other cancers. Satraplatin and other platinum-based drugs are known for undergoing chemical transformation in the blood steam but the role of haemoglobin in this process is unclear. In the present study, we characterised the reactions occurring between satraplatin and haemoglobin *in vitro*. Methods: The satraplatin concentration of reaction solutions was determined by a satraplatin-specific HPLC-ICPMS assay. The stability of satraplatin was determined in haemoglobin solutions under various conditions by changing the ligandation and oxidation state of haemoglobin, availability of co-factor and other experimental variables. Results: In solutions containing met-haemoglobin, satraplatin was completely stable. However, in solutions containing reduced haemoglobin and NADH, satraplatin was rapidly converted ($t_{1/2}$, 35 mins) to its major Pt(II) active metabolite (JM118). Visible spectrometry of the reaction solution revealed increasing absorption at 500 and 630 nm and decreasing absorption at 542 and 576 nm, characteristic of the conversion of oxy-haemoglobin to met-haemoglobin. Factors affecting the reaction between satraplatin and haemoglobin were investigated. Cold temperature, carbon monoxide, oxygen and the absence of co-factor inhibited the haemoglobin-catalysed metabolism of satraplatin. Conclusion: Haemoglobin catalysed the activation of satraplatin by a mechanism involving electron transfer from haem-iron to drug molecules, resulting in the reduction of the parent Pt(IV) drug to active Pt(II) metabolites concurrently with the oxidation of haeme iron from Fe(II) to Fe(III). Inhibitor studies suggested that reduced unliganded haem-iron is required for the haemoglobin-catalysed metabolic activation of satraplatin.
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Histone deacetylase inhibitors induce chromatin decondensation by down-regulation of proteins involved in the maintenance of heterochromatin

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Background: Genome stability and transcriptional silencing are accomplished by condensation of the chromatin in the form of heterochromatin. The maintenance of heterochromatin is regulated by hypermethylation and hypoacetylation of DNA and histones. Histone acetylation attenuates the electrostatic interactions between histones and DNA. This may loosen the chromatin structure. This study evaluates the effects of histone deacetylase inhibitors (HDACi) on chromatin structure and dynamics and investigates a mechanism by which HDACi may increase the susceptibility of cancer cells to DNA damaging agents.
Methods: Changes in the chromatin structure were evaluated by electron microscopy, DNA hypersensitivity assays, and DNA binding assays. The effects of HDACi on protein expression were evaluated using microarray, Northern and Western blot experiments.
Results: We found that exposure of breast cancer cells to the HDACi, valproic acid, suberoylanilide hydroxamic acid, sodium butyrate and trichostatin A resulted in histone hyperacetylation and chromatin decondensation. While maximal histone acetylation occurred within one hour, chromatin decondensation required prolonged exposure to an HDACi for at least 48 hours, suggesting that histone hyperacetylation alone may not be sufficient for chromatin decondensation. We therefore evaluated the effects of HDACi on other factors involved in the epigenetic control of chromatin dynamics. A down-regulation in the expression of several family members of the structural maintenance of chromatin (SMC) proteins and SMC-associated proteins, DNA methyltransferase and heterochromatin proteins was observed after 48 hours exposure to an HDACi. This correlated with enhanced sensitivity of DNA to nucleases and increased interaction of DNA with intercalating agents. The HDACi-induced chromatin decondensation correlated with enhanced DNA damage and apoptosis induced by DNA damaging agents such as topoisomerase I and II inhibitors.
Conclusions: HDACi-induced histone hyperacetylation alone is not sufficient for chromatin decondensation, but is associated with the down-regulation of gene products involved in the maintenance and dynamics of heterochromatin. The HDACi-induced chromatin decondensation may facilitate access of DNA damaging agents to their target sites and thereby enhances their efficacy.

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Evidences for G-quadruplex formation in the mitochondrial genome

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G-quadruplex (G4) DNA sequences, as found in human telomeres and certain oncogenes (e.g. c-*myc*), are considered as potential targets for anticancer agents. Various G4 sequences have been identified in the nuclear genome. Here we show that such folded sequences can also be found in the mitochondrial genome. Mitochondrial DNA (mtDNA) is a compact circular double-stranded DNA of 16.6 kb with few intergenic sequences and no intron, and presents G rich (Heavy) and C rich (Light) strands. mtDNA mutations have been characterised in various types of human cancers and these mutations may contribute to carcinogenesis or tumor cell proliferation. It is therefore important to identify regulatory sequences in the mtDNA. In our study, G-rich oligonucleotides corresponding to defined regions in the mitochondrial genome were evaluated for their ability to form G-quadruplex structures. Using different spectroscopic approaches, we demonstrate that two oligonucleotides, Mt2 and Mt4, were able to form G-quadruplexes which stability depends on the nature of the mono-cation. We also investigated the binding of the bis(quinacridine) macrocyclic compound BOQ1 to these sequences by electrospray mass spectrometry and fluorescence studies. We show that this compound, previously described as a G-quadruplex telomeric DNA binding molecule, interacts selectively with the two quadruplexes Mt2 and Mt4. The identification of different regions in the mitochondrial genome potentially capable to form G-quadruplexes provides opportunities to target these sequences with small molecules.