**Conclusions:** We employed *in vivo* phage display in human prostate tumor-bearing mice to identify peptides that extravasate the vasculature and specifically target the tumor cells, not just the surrounding vasculature. This finding is significant because previous *in vivo* selections preferentially retrieved phage that bound vascular components (Arap, Nat Med 8:121). Thus, our studies may facilitate the development of a range of cancer cell-surface or internalizing molecules not previously realized.

**49** POSTER

**DNA aptamers that recognize the MUC1 tumour marker**

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Mucins are high molecular weight glycoproteins that provide a protective layer on epithelial surfaces and are involved in cell-cell interactions, signalling, and metastasis. The membrane-bound MUC1 mucin is expressed in normal mucosas and the aberrant expression of its under-glycosylated forms has been reported in various carcinomas of the epithelium. MUC1 is a human tumour antigen expressed in breast, pancreatic and ovarian cancers. Agents able to bind tightly and specifically to the surface of malignant cells would greatly benefit cancer diagnosis and treatment, whereas the targeting of cell surface receptors would have significant implications on inflammation and immunity. While antibodies have the ability to specifically recognise some tumour cell markers, their large size and own immunogenicity markedly limit their pharmacological value. The development of nuclease resistant DNA molecules, termed *aptamers*, has provided a new alternative to antibodies. Using the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) methodology, one can generate vast libraries of oligonucleotide ligands (DNA, RNA, or unnatural products), screened rapidly for specific sequences that have appropriate binding affinities and specificities to the clinically relevant marker.
We have identified synthetic DNA oligonucleotide aptamers that bind to the MUC1 tumour marker with low nanomolar affinity via the 20 tandem repeat sequence of the MUC1. These specific aptamers were selected from an initial library that contained a degenerate region of 25 bases to result in $4^{25}$ random-sequence DNA molecules. Ten rounds of in vitro selection and amplification were performed, to confer affinity maturation of aptamers for MUC1. Selected aptamers were cloned, sequenced and found to be sharing some unique consensus sequences. The affinity of each aptamer for MUC1 was studied by qualitative and quantitated methods such as ELISA, BIAcore, and EMSA. Affinities on the nanomolar range have been identified and confirmed.
Efforts in our laboratory are now focusing on optimization of their delivery, functionality and structural properties. Fluorescent labelled aptamers have been successfully used in the identification of MUC1 expressing tumour cell lines such as the MCF7 breast tumour cell line and can be used in future diagnostic assays, whereas radiolabelled aptamers can be used clinically to enable imaging and therapy of the tumour marker bearing cancer cells.

**50** POSTER

**Inhibition of histone deacetylase 2 increases apoptosis and P21 expression**

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**Background:** Histone deacetylases (HDACs)1 and 2 share a high degree of homology and are known to co-exist within the same protein complexes containing other transcriptional co-repressors. Despite their close association, studies have shown that each possesses unique functions, which cannot be compensated by the other. This study describes the regulation of HDACs1 and 2 in colorectal and cervical cancers and the function of HDAC2 in apoptosis.
**Materials and Methods:** A combination of quantitative RT-PCR and tissue array was used to determine the expression of HDAC1 and HDAC2 in colorectal cancer and matched normal mucosal samples. Immunohistochemistry was used to determine the expression of HDACs1 and 2 in cervical cancers and dysplasias. HDAC2 expression in HeLa cells were knocked-down by specific siRNAs designed against HDAC2 (Dharmacon Inc). The efficacy of knock-down was confirmed by Western blot analysis. Apoptosis was assayed by annexin V-FLUOS staining and flow cytometry. P21 expression was determined by Western blot analysis using a specific antibody (Snata Cruz Biotechnologies).
**Results:** Both HDACs1 and 2 are upregulated at the mRNA (n=16) and protein (n=45) levels in colorectal cancer. The upregulation of HDAC2 was more robust and occurred more frequently in the samples. It also occurred early in the carcinogenic process, with 4 of the 5 polyps showing upregulation of HDAC2 compared to normal mucosa. In cervical carcinoma (n=9), the expression of both HDACs1 and 2 was correlated with the severity of cervical dysplasias and invasive carcinomas of the cervix. However, HDAC2 expression showed a clear demarcation of higher intensity staining at the transition region of dysplasia. Further, more cells were stained for HDAC2 than HDAC1 in cervical dysplasia. The functional significance of HDAC2 upregulation was determined by knocking down the expression of HDAC2 with HDAC2-specific siRNA. Cells displayed an increased number of cellular extensions reminiscent of cell differentiation after HDAC2 knockdown. There was also an increase in apoptosis, associated with an increase in P21 expression.
**Conclusion:** The results suggest that histone deacetylases, especially HDAC2, are important enzymes involved in the early events of carcinogenesis, making them candidate markers for tumor progression and targets for cancer therapy.

**51** POSTER

**HSV-tk gene transduction enhances proliferation rate and COX-2 expression in rat gliosarcoma cells**

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**Background:** Transduction of tumor cells with the Herpes Simplex Virus thymidine kinase (HSV-tk) gene and consequent treatment with ganciclovir is widely used for suicide gene therapy of brain tumors. Recently we observed that HSV-tk gene transduction of rat gliosarcoma (9L) cells enhances the expression of cyclooxygenase-2 (COX-2) and the release of prostaglandin $E_2$ ($PGE_2$). It is well established that COX-2 overexpression with increased production of COX-2-derived prostaglandins are associated with multiple aspects of carcinogenesis, including the control of cell proliferation and increased resistance to apoptosis and chemotherapy.
**Aims:** In the current work we aimed to: a) determine the effect of HSV-tk gene transduction on proliferation rate of 9L cells; b) evaluate the effect of nimesulide (NIM), a selective COX-2 inhibitor, on proliferation rate of wild-type (9L) and HSV-tk transduced (9L/HSV-tk) 9L cells.
**Results:** Western blot analysis of COX-2 protein expression in 9L and 9L/HSV-tk cells showed that COX-2 is overexpressed in HSV-tk transduced cells, while wild-type cells did not express COX-2 at detectable levels. COX-2 overexpression in HSV-tk transduced cells was accompanied by increased release of $PGE_2$, assessed by radioummunoassay, into the culture medium ($2.5\pm$ 0.2 *vs* $102.3\pm9.4$ ng/$10^6$ cells for 9L and 9L/HSV-tk cells, respectively). In order to determine the effect of HSV-tk gene transduction on cell proliferation rate, 9L and 9L/HSV-tk cells were incubated for 120 hrs in 24-well culture plates and the number of attached cells was counted every 24 hrs. We found that proliferation rate of 9L/HSV-tk cells was 2 to 3-fold higher, compared to that of wild-type 9L cells. To evaluate whether increased release of $PGE_2$ accounts for the observed enhancement in proliferation rate in the 9L/HSV-tk cells, we investigated the effect of NIM on proliferation rate of both wild-type and HSV-tk transduced cells. Incubation of 9L and 9L/HSV-tk cells with NIM at a concentration which inhibits COX-2 activity, completely abolished $PGE_2$ release in both wild-type and HSV-tk transduced cells for as long as 96 hrs. However, at this concentration, NIM failed to affect proliferation rate of 9L and 9L/HSV-tk cells.
**Conclusions:** Taken together, we demonstrate herein that HSV-tk gene transduction enhances proliferation rate and COX-2 protein expression and activity in rat gliosarcoma cells. Additionally, the enhanced proliferation rate of HSV-tk transduced cells appears to be independent on prostaglandins overproduction since treatment with the selective COX-2 inhibitor failed to abolish the enhancement of the proliferation rate.

**52** POSTER

**Attenuated immunogenicity and toxicity of PEGylated recombinant methioninase (PEG-rMETase) in primates**

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**Background:** Methionine depletion by recombinant methioninase (rMETase) has been demonstrated to be an effective antitumor regimen in tumor-bearing mouse models. However, the therapeutic potential of rMETase has been limited by its short plasma half-life and immunological effects, including high antibody production in mice and monkeys and anaphylactic reactions in monkeys.
**Materials and Methods:** In order to improve the therapeutic potential of rMETase, a PEG-rMETase conjugate has been developed by coupling the enzyme to methoxypolyethylene glycol succinimidyl glutarate (MEGC-PEG-5000). In this study, we evaluated the pharmacokinetics, antigenicity and