identified in unaffected children at risk. C-cell hyperplasia was found in 4 children (13, 10, 9, and 8-year-old) with elevated levels of stimulated plasma Ct who underwent surgery. In the 8-year-old child mutation carrier with HD and normal CT values, histological examination did not demonstrate MTC or C-cell hyperplasia. Although the association of MEN 2A with HD is infrequent, the occurrence in this family of both phenotypes with a single mutation of the RET protooncogene, suggests that HD should be considered as part of the MEN 2A disease spectrum, and screening for MTC and pheochromocytoma is indicated in patients with HD associated with mutations in exon 10 of the RET protooncogene.

Our data indicate that genetic screening of MEN 2A pedigrees allows the early identification of germ-line mutation in the RET proto-oncogene and suggest a prophylactic thyroidectomy in gene carriers, as early as possible, before the development of biochemical or clinical evidence of MTC.

Loss of P16 protein expression correlates with tumour progression in cutaneous melanoma

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Introduction: Inactivation of the p16 tumour suppressor gene has been reported frequently in melanoma cell lines and mutations have been detected in familial melanoma kindreds. The aim of this study was to assess the role of p16 inactivation in melanocytic progression by measuring the level of p16 protein in a range of sporadic benign and malignant melanocytic lesions.

Methods: Using dual parameter flow cytometry p16 protein expression was measured in 30 benign melanocytic naevi (BMN), 38 primary and 51 metastatic melanomas.

Results: A high level of p16 expression was demonstrated in BMN (96% median nuclear positivity) with a significant reduction when compared to primary melanomas (69%, $P<0.001$). The median nuclear positivity of primary melanomas was significantly higher ($P<0.03$) than the level of expression in metastatic lesions (median positivity 37%). Ulceration of the primary tumour showed an association with p16 expression. Median p16 positivity for non-ulcerated tumours was 79% (range 68–86%) compared to 59.5% for ulcerated primaries (range 32–89%). This difference in positivity was found to be statistically significant ($P=0.02$). A similar trend was observed when comparing p16 expression with the number of mitotic figures. Significantly lower immunopositivity was observed in tumours with increased mitotic figures (median 58%, range 32–76%) compared to primaries with scanty mitoses (median 73%, range 36–89%, $P=0.04$%).

Conclusion: A progressive loss of p16 expression was demonstrated from BMN through to primary and metastatic lesions. These data suggest that loss of p16 protein expression is associated not only with the early transformation of benign lesions but also in the later stages of malignant progression. The association of ulceration and prominent mitoses with low p16 expression fit with the putative role of p16 as a tumour suppressor in control of the cell cycle during the G1 check point.

Drug resistance evaluation by functional assay and immunofluorescence in bladder cancer

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Superficial bladder cancer frequently recurs and can evoke repeated chemotherapy. However, multidrug resistance (MDR) limits the effectiveness of chemotherapy.

We assessed MDR in recurrent bladder cancer ($n=11$) by (i) immunofluorescent staining of three proteins associated with MDR, P-glycoprotein (P170), MDR related protein (MRP) and lung resistance protein (LRP) in frozen sections and (ii) by confocal microscopy of epirubicin accumulation (20 mg/ml, 2 hours) in primary explants in cultures. Specifically, intracellular drug distribution was determined including changes under the influence of the MDR reversing agents, verapamil and PSC833. Five cell lines (including resistant variants) were used for method development and afforded the 'gold standard' results for both assays.

7/11 specimens exhibited positive immunofluorescence; 5 for P170 with or without MDR proteins, 1 for MRP and 1 for LRP, with the percentage of stained cells usually <15%. Of the 7/11 immunohistochemically resistant cancers, only 5 showed a resistant pattern of epirubicin uptake in culture; ie, lesser amounts of total drug uptake compared to sensitive cultures (3x–10x), nuclear exclusion of drug, reversal of the latter pattern using verapamil and/or PSC833. Of the 4/11 specimens with negative immunofluorescence, 2 grew resistant explants *in vitro*. Overall, only 7/11 specimens gave matching results using both assays (sensitive/sensitive or resistant/resistant).

These early results show that both immunofluorescence and functional assay give information on MDR status. The majority of tumours showed at least small populations of resistant cells. The clinical implication is that reversal strategy should be developed for early or prophylactic implementation.

MDR = Multidrug resistance, P170 = P-glycoprotein, MRP = Multidrug resistance related protein, LRP = Lung resistance protein.

Suicide gene therapy for colorectal liver metastases: evidence of a systemic anti tumor immune response

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Expression of the HSV1-TK gene in localized tumours results in tumour regression following the administration of ganciclovir. A localized anti tumour immune response contributes to this regression. The aim of this study was to determine if the treatment of localized HSV1-TK expressing colorectal liver metastases resulted in a systemic anti tumour immune response and the regression of distant metastases. The effect of HSV1-TK expression in the absence of drug selection was also investigated. Liver metastases were generated by the subcapsular injection of 5×10^6 cells of the K12TK cell line (Groups 1 and 2) and K12 cell line (Group 3) in BDIX rats. All animals received a second injection of the K12 cell line in an adjacent lobe of the liver. After 7 days, Group 2 were treated with intraperitoneal GCV 755 mg/kg b.d. for 5 days. Following sacrifice tumour volumes were determined and T cell cytotoxicity assays performed. HSV1-TK expressing tumours showed marked regression following GCV (Group 2), mean tumour volume 0.6 mm^3 ($P<0.0001$, Mann–Whitney) compared to 98.4 mm^3 for controls (Group 3). However, HSV1-TK protein expression alone also resulted in tumour regression (Group 1), mean tumour volume 22.4 mm^3 ($P<0.05$). Adjacent tumours in Group 2 had a mean tumour volume of 45.2 mm^3 (NS), whilst Group 1 showed significant regression, mean tumour volume 20.2 mm^3 ($p<0.05$). A cytotoxic T cell population recognizing the umodified K12 cell line was identified in Group 2.

Conclusions: HSV1-TK mediated therapy of experimental liver metastases results in a systemic anti tumour immune response. The TK protein appears to have a role in the generation of anti tumour immunity.

HSV1-TK: Herpes Simplex Virus Type 1 Thymidine Kinase.

GCV: Ganciclovir.

Experiments were performed under the Animals (Scientific Procedures) Act, 1986.

Breast Cancer

New stereotactic excision of non-palpable mammographic abnormalities: the ABBI system

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Screen detected suspicious or malignant mammographic abnormalities have traditionally been managed by wire localisation and excision. This has associated morbidity due to the need for general anaesthesia, excision of significant breast tissue and the requirement for multiple procedure in some patients.

We present our initial experience with the ABBI (Advanced Breast Biopsy Instrumentation) system (Auto Suture International, USSC, Norwalk, USA), used for core biopsy, diagnostic and therapeutic procedures in-patients with screen detected mammographic abnormalities. All procedures were done under local anaesthesia as day cases.

Fifty-seven patients with screen detected abnormalities were evaluated with this system. Fourteen (14) patients underwent ABBI excision biopsies:

HNSCC = Head and neck squamous cell carcinomas; OSCC = oral/oropharyngeal squamous cell carcinomas; STRP = short tandem repeat polymorphisms.

The midfacial degloving approach to sinonasal tumours

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The midfacial degloving approach (MFD) provides wide access to the nose sinus's nasopharynx and skull base. It avoids facial scars and facilitates bilateral surgery. It also avoids the risk of post-operative palatal dysfunction.

Fifty two such procedures have been performed at the SUNY ORL Department in an eight year period.

These include 42 patients with midfacial and skull base tumours including inverted papilloma, carcinoma, angiofibroma and esthesioncuroblastoma. The age range was 4–82 years.

In all cases adequate exposure was obtained to permit good local clearance.

The main complication was paraesthesia in the distribution of the infraorbital nerve, although usually short-lived, this persisted for one year in three patients. There was one case of symptomatic post-operative nasal stenosis.

The MFD approach is now the procedure of choice for angiofibroma and the the en-bloc removal of tumours of the antro-ethmoidal region when full craniofacial resection is not required.

Posters

MRI in the detection of breast cancer multicentricity

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The significance of small multicentric foci in breast cancer has been questioned. Whilst mammograms rely on microcalcifications for detecting cancers, contrast-enhanced MRI relies on vascularity and vascular permeability. We propose therefore that MRI would detect only clinically important multicentric foci. In this study we compared the pre-operative detection of multicentric foci by contrast-enhanced MRI with standard radiological-histological examination of the resected specimen.

Method: Ten patients with newly diagnosed breast cancer underwent preoperative contrast-enhanced breast MRI using a transverse T1-weighted three dimensional (3D) FLASH sequence. After surgical excision the specimens were fixed and cut in the same plane as the MRI. The pathologist sampled any identifiable lesion for histological analysis and subsequently a mammogram was performed on the specimen slices. Two observers identified calcifications and these were then sampled and paraffin blocked. One section was cut from each block, stained and examined by microscopy. The remaining blocks were x-rayed and any calcifications led to further sectioning. MR images were reviewed independently and findings compared with histology.

Results: On mammography of tumour slices, 71 suspicious areas were identified and sampled. Histologically, 12 areas of DCIS or invasive cancer were identified 1–4 cm from the primary tumour. Two tumours showed diffuse patchy enhancement which on histology was DCIS. There was concordance between the magnitude of satellite enhancement foci and the number of histologically abnormal samples.

Conclusion: These preliminary findings suggest that MRI is highly sensitive for showing multifocal invasive or in-situ tumours but that the specificity for the diagnosis of malignant change in these foci may be low.

Expression of antimetastatic oncogene nm23 in colorectal carcinoma definition with P.C.R.

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The gene nm23 is located in the chromosome 17,q2 11.3-22, and its expression is associated with low metastatic potential.

The aim of the study was to demonstrate the value of nm23 as a metastatic potential indicator. For this purpose selected cases of colorectal carcinomas (blood serum and tissue samples from paraffin blocks were obtained) were divided in two groups: five cases with low infiltrating tumors and five cases of tumors with local and distant invasion. We measured 1) the expression of nm23 monoclonal antigen in paraffin blocks using immunohistochemical methods and 2) the transcriptional levels of antigen in blood serum samples using molecular biology techniques.

The resulting analysis demonstrated an increased expression of nm23 (in both tissue and blood serum samples) in the cases with low infiltrating tumors and absence of any expression in tumors with local or distant metastasis. Low differential carcinomas had no detectable expression of the oncogene nm23, while the physiological colorectal mucosa (obtained from the same paraffin blocks) expressed low levels of the nm23.

In conclusion, it seems that there is a strong correlation between the expression of the nm23 and the metastatic potential in colorectal carcinoma and probably measurement of nm23 levels may be an important indicator of the biological behaviour of this type of tumor.

Assessment of cellular markers in oral tumours

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Aim: To assess markers of apoptosis and cellular proliferation and to determine whether these are linked to prognosis in a highly selected group of patients.

Method: The patient groups were selected from our clinical database and included:

a) the five most clinically indolent tumours.
b) the five most clinically aggressive tumours.
c) a group of four tumours, where a good long term clinical outcome was noted despite extensive nodal involvement. Material was tested using bcl 2 as a marker of apoptosis, mib 1 as a marker of cellular proliferation and also p53. Slides were prepared from archived material and immunohistocytochemistry staining performed by the streptavidin biotin complex method on a DATO Techmate 500 processor. The specimens were also tested for high risk HPV sub-types by PCR.

Results:

a) None of the tumours were positive for bcl 2.
b) There was no correlation between clinical aggressiveness and cellular proliferation as measured by mib 1.
c) P53 likewise did not correlate with tumour aggressiveness.
d) HPV positive tumours were only seen in the indolent group.

Conclusions: Using standard markers, no relationship between apoptosis and cellular proliferation and tumour behaviour has been demonstrated in this small series. However, only tumours with an indolent clinical behaviour were positive for HPV and this possible relationship merits a larger study.