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Cancer pathology in the year 2000

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Abstract

The last one hundred and fifty years has produced the mature and sophisticated discipline of histopathology, yet still leaves the diagnosis of human cancer, by the best available technique, as more art than science. Proton magnetic resonance spectroscopy (^{1}H MRS) ex vivo identifies the chemical markers of established pathobiological disorders within excised biopsies and fine needle aspirates, in particular, those associated with the development and progression of malignant disease. Alterations to cellular chemistry monitored by ^{1}H MRS allow distinction between invasive and pre-invasive lesions of the uterine cervix [1], and separate truly benign follicular neoplasms from follicular carcinomas on analysis of fine needle aspirates containing as few as 10^6 cells [2,3]. ^{1}H chemical shift imaging (CSI) determines the spatial location of these chemical changes and provides insight into the chemistry of neoplastic transformation [4,5]. It is our hypothesis that, by the year 2000, CSI will aid image guided biopsy techniques and that correlation of biopsy histology with in vivo localised ^{1}H MRS data will: (a) lead to improved assessment of the extent of malignant disease and (b) establish the sensitivity and specificity of in vivo ^{1}H MRS for the simultaneous determination of the size, location and neoplastic potential of a tumour mass. © 1997 Elsevier Science B.V.

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1. Introduction

The use of magnetic resonance imaging (MRI) to discriminate between normal and malignant tissue on the basis of the relaxation rates of water signals was proposed by Damadian over twenty years ago [6]. Although water-based MRI technology has dramatically improved since then, its usefulness in cancer detection has been disappointing. Whilst MRI has in some cases been able to identify the location and extent of lesions and to some extent infer the malignant potential by identifying non-specific features, it has not been able to precisely determine the pathology and hence the clinical outlook for the patient [7]. For this to be achieved, a biopsy is still required and the histopathologist given the opportunity to view the morphology of the tissue.

MRI was first realised to be ineffective for determining the pathology of tumours due to the great variability in the MR profiles of normal healthy tissues. Furthermore, during the 1980s, it was not known that chemical species other than water made a contribution to the tumour images. More pertinently, the value of the panopoly of diagnostic molecules resonating under the intense water signal was yet to be appreciated. At least 50 different chemical species are currently measurable simultaneously by ^{1}H MRS

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during tumour development and progression. However, the potential use of these chemical signatures for diagnostic purposes has not been investigated in patient cohorts sufficiently large to accommodate thorough statistical analysis [8].

^{1}H MRS enables an assessment of the chemical composition of cells and the changes which occur with disease. We have now established that ^{1}H MRS (ex vivo) can provide an adjunct to, and in some cases replace, current cytological methods for the diagnosis of human tumours ex vivo with sensitivities and specificities of or above 94% for cervix [1], colon [9], ovary [10], breast [11] and thyroid [2,3]. The acute sensitivity of the MRS method was illustrated in the detection, in tumour-bearing rats, of microscopic metastatic deposits in lymph nodes [12]. These micro-metastases were missed by histopathological techniques but confirmed by xenografting the nodes into nude mice and recording subsequent tumour growth.

^{1}H MR chemical shift imaging (CSI) is able to determine both the alterations to cellular chemistry and the spatial location of diseased tissues by mapping variations in the relative concentrations of different metabolites. The chemical criteria first determined by MR spectroscopy to distinguish between normal, benign, pre-invasive and frankly invasive carcinomatous tissue, have now been used for chemical shift micro-imaging ex vivo [4,5]. Others around the world have shown CSI data are obtainable directly, in vivo [13].

Magnetic resonance has the capacity to take the art out of pathology and surgery. The clinical application of magnetic resonance (MR) to the detection, diagnosis and management of cancer has been slow to emerge, due mainly to a lack of knowledge of those MR measurable chemicals which are diagnostic. Furthermore, over the last twenty years, following the report by Fidler and Kripke of tumour heterogeneity [14], it has become evident that tumour development and progression is a continuum of chemical changes reflecting a variety of biological events. By comparison of ^{1}H MR profiles with histopathology (the current 'gold standard') and clinical outcome, it has now been demonstrated that different chemical species are diagnostic for each organ and pathological state [8] and that MR visible chemistry reflects the biological events in progress.

2. The future of MR in the cancer clinic

Diagnosis of human cancer and its subsequent management are currently dependent upon the interaction of a number of highly trained medical specialists. Too often, during the interaction of these medical staff, the individual needs of specific patients are translated into subjectivity. Removing this subjectivity bias, a role we see as being played by MR technology, will impact positively on the process overall, particularly accuracy of diagnosis and premanagement assessment.

It is our hypothesis that in cancer clinics in the year 2000, ex vivo diagnosis of human tumours will be conducted by laboratory scientists, in real time, on tissue or cellular samples. Small bench-top diagnostic magnets will be available for such purposes, operated by laboratory scientists under the direction of the pathologist. Confirmation and 'fine tuning' of the diagnosis will be done on the same or parallel tissue/cellular samples in the usual histopathological way. An immediate confirmation or exclusion of the diagnosis of malignancy will thus be available for accurate and efficient triaging of patients, management decisions and counselling.

3. Uterine cervix

Cancer of the uterine cervix is the second commonest malignancy in women worldwide [15]. Screening programs to decrease the incidence of cervical cancers are based on the premise that they are preceded by pre-invasive neoplastic changes described as cervical intraepithelial neoplasia (CIN) or, more recently, as squamous intraepithelial lesions (SIL).

Diagnosis of cervical cancer currently relies on histological examination of the tissue obtained during colposcopy and this process is not free of problems. Sampling errors, by the gynaecologist, can be introduced at the time of biopsy, to be compounded by processing artefacts in the laboratory and subjective assessment of the sections by the pathologist. By definition, the in situ phase of an epithelial malignancy contains cells which are morphologically indistinguishable from those in the invasive state. Diagnosis of invasive cancer therefore rests, not on

cytological criteria, but on histological evidence of destructive invasion.

3.1. Spectroscopy

1D ^{1}H MR spectra were obtained from 40 specimens of invasive carcinoma and 119 preinvasive specimens [1]. Spectra of 39/40 invasive specimens (Fig. 1B) were characterised by an intense resonance at 1.3 ppm arising primarily from methylene protons of acyl chains in mobile neutral lipid with additional contributions from the methyl protons of lactate and threonine.

The spectra of high grade carcinoma in situ (*CIS*)/*CIN3* specimens (Fig. 1A) were similar with resonances arising from the methyl, $^+N(CH_3)_3$ and olefinic resonances at 0.9, 3.2 and 5.2 ppm, respectively. In addition, there were prominent resonances at 1.7, 2.0 and 3.0 ppm but preinvasive specimens lacked the intense methylene resonance at 1.3 ppm. The other obvious spectral difference was an increase in the broad featureless resonances between 3.4 and 4.2 ppm in pre-invasive specimens relative to their invasive counterparts. This latter resonance which arises mainly from protons on carbohydrate, protein and phospholipid metabolites (marked on Fig. 1A by an arrow), is denoted as the CH resonance. When the CH_2/CH_3 ratio, was plotted against the CH/CH_2 ratio comparing the premalignant states to invasive carcinoma, a separation between invasive and preinvasive was achieved with sensitivity and specificity of 94% and 98%, respectively [1].

Thus, ^{1}H MRS was able to distinguish between pre-invasive and invasive cervical cancer ex vivo, based on the detection of altered cellular chemistry in invasive cells. The problem of defining invasive versus non-invasive carcinoma can thus be overcome with use of a simple MRS experiment which leaves the biopsy intact for subsequent further histological assessment [1]. An independent study undertaken by the National Research Council in Winnipeg substantiates these data [16,17].

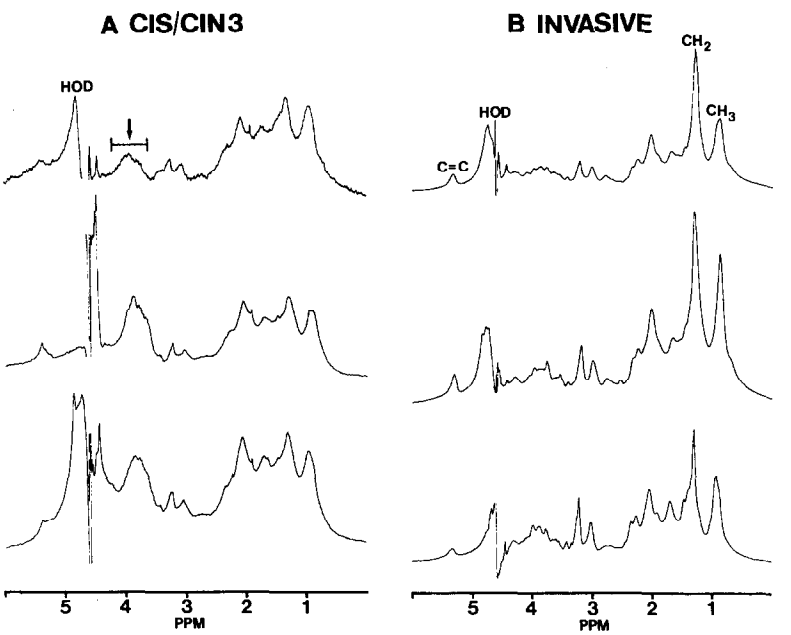

Fig. 1. 1D ^{1}H MR spectra of cervical punch biopsies. Three examples of each of the following histological classifications are shown. (A) CIS/CIN3 and (B) invasive carcinoma. Spectra were recorded on a Bruker AM-360 MHz spectrometer at 37°C with the sample spinning. The number of accumulations was 256 and a line broadening of 3 Hz was applied before Fourier transformation. Residual water was suppressed by gated irradiation.

3.2. Chemical shift imaging

The clear discrimination between pre-invasive and invasive cervical disease based on the appearance of a lipid signal, indicated the potential for CSI to provide both the location and the nature of a cervical lesion simultaneously. Water and lipid-based (excitation centred at 4.8 and 1.2 ppm, respectively) MR images were obtained from a biopsy containing foci of carcinoma in situ (CIS) and a different biopsy of an invasive cervical cancer placed in a single 5 mm sample [4]. A typical result of the CSI experiment is shown in Fig. 2A (water image) and 2B (lipid image) and compared with the low power (Fig. 2C) and high power (Fig. 2D) histology. The two biopsies are outlined in the water-based image (Fig. 2A). The bottom half of the image is occupied by a biopsy with invasive cervical adenocarcinoma, while a biopsy with squamous CIS is in the top half of the image. In the lipid image (Fig. 2B), the distinction between invasive and pre-invasive cervical neoplastic epithelium was apparent. Bright areas were de-

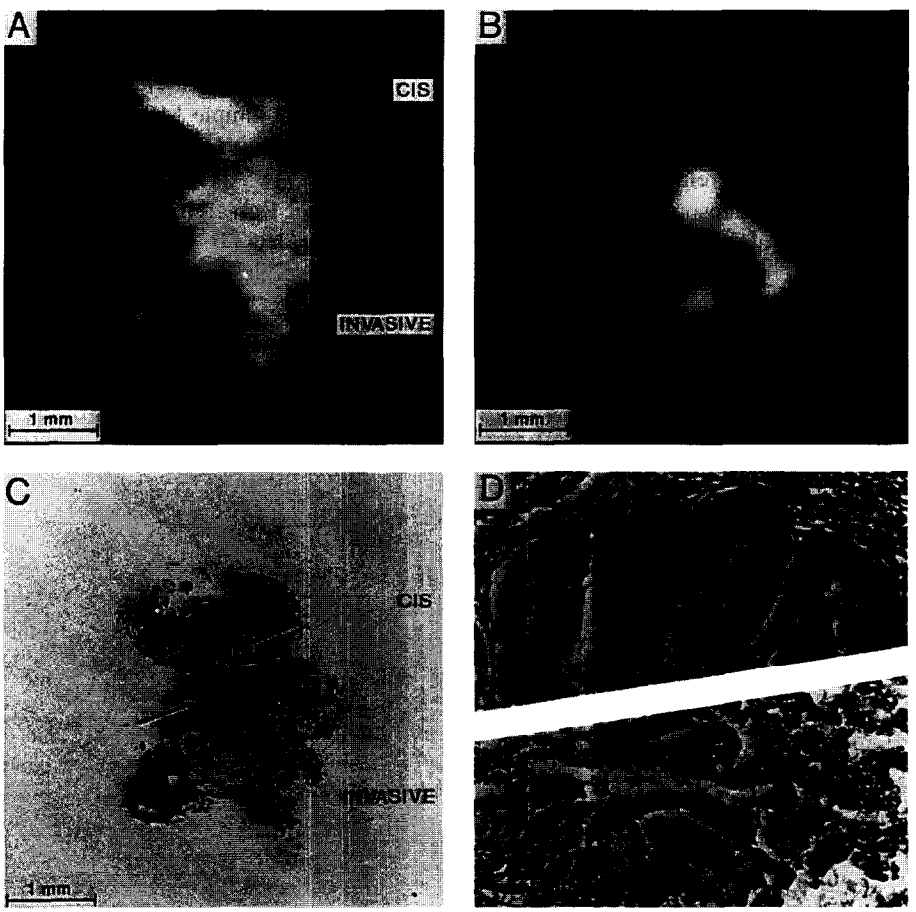

Fig. 2. MR CSI allows both the location and pathology of a cervical lesion to be obtained simultaneously. Water based (A) and lipid based (B) images of two human cervical punch biopsies are shown. The lower biopsy is an invasive carcinoma and the upper biopsy is a CIS/CIN3. Distinction between the two pathologies is apparent, with bright areas detected in the lipid image of invasive carcinoma but not CIS/CIN3. A Gaussian filter (A, 10 kHz and B, 5 kHz at half height) was applied in both imaging directions prior to Fourier transformation. (C) A 5 μm histological cut through the two biopsies parallel to and at the centre of the imaged slice. (D) Magnification ($\times 10$) of the tissue area indicated by the arrows in (C). The top and bottom panels show the histology of the CIS and the invasive carcinoma, respectively.

tected only from the lower biopsy of invasive carcinoma.

The transition from CIS to overtly invasive cervical cancer is accompanied by the emergence of strong signals from MR-visible lipids. The network of intense lipid signals in the lipid-based images was shown to coincide with foci of cytologically malignant and invasive cells. Within these bright areas, chemical shift images show local 'hot spots' corresponding to a pronounced increase of MR-lipids. Histologically, there was no increased concentration of malignant cells at these hot spots compared to the directly adjacent invasive tissue with normal brightness in the image. While others have shown that droplets of neutral lipid accrue in hypoxic regions of fast growing tumours [18], suggesting that necrosis could cause the hyper intense areas detected by CSI, no regions of necrosis were apparent in the corresponding histological section of the cervical tissue. We therefore postulate that the hyper intense areas in the CSI of carcinoma of the cervix reflect areas of increased biochemical activity.

4. Thyroid

Thyroid nodules are common and are estimated as being clinically evident in up to 10% of the population. While the vast majority (90–95%) of solitary thyroid nodules are benign [19,20], the exclusion of malignancy in follicular thyroid nodules remains a significant diagnostic problem, made currently on the excised lesion obtained at a thyroidectomy. Pre-operative fine needle aspiration biopsy (FNAB) cytology, although accurate in identifying papillary, medullary and anaplastic carcinoma, is unable to distinguish benign from malignant follicular neoplasms.

Histologically, tumours that are arbitrarily designated as follicular adenomas and follicular carcinomas are indistinguishable in clinical, radiological and gross pathologic features, relying on detection of capsular or vascular invasion at the periphery of the neoplasm to identify the carcinomas. As noted above, this requires surgical removal of the entire tumour and extensive laboratory examination.

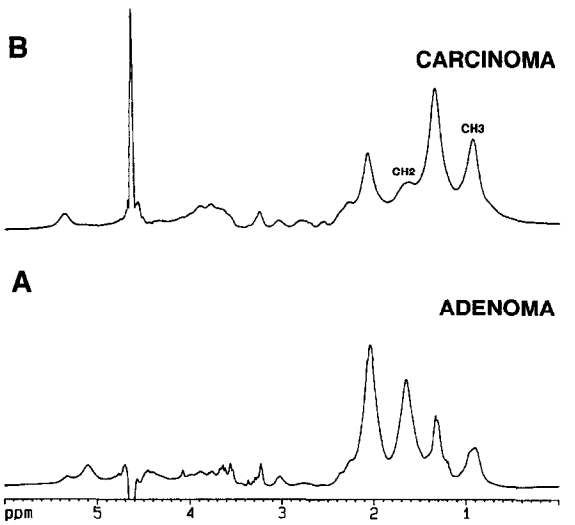

Fig. 3. 1D ^{1}H MR spectra of (A) follicular adenoma and (B) follicular carcinoma. Spectra were recorded on a Bruker AM-360 MHz spectrometer at 37°C with sample spinning. The number of accumulations was 256 and a line broadening at 3 Hz was applied before Fourier transformation. Residual water was suppressed by gated irradiation.

4.1. Spectroscopy

In a study of 53 consecutive patients with thyroid nodules, we established that 1D ^{1}H MRS could distinguish normal thyroid tissue from proven carcinoma of all types with a sensitivity and specificity of 100% ($p < 0.0001$, Student t-test) [2]. Examples of 1D spectra from follicular adenoma and follicular carcinoma are shown in Fig. 3. The basis of the discrimination was altered cellular chemistry reflected in the resonance intensity ratio at a chemical shift of 1.7 ppm (arising from lysine) and lipid at 0.9 ppm. The lipid spectral profile is much weaker in adenoma than carcinoma showing the same trend previously observed for uterine cervix [1].

Histopathologically benign follicular lesions, as currently defined, were found to span both the normal and malignant spectral patterns. The biological significance of the span of MR spectral ratios recorded for follicular lesions is unclear, although it would support the concept that there is, in fact, a gradual progression from benign to malignant, preceding any histological evidence of malignancy. Molecular genetic studies have recently supported this concept [21].

4.2. Fine needle aspiration biopsies (FNAB)

Cytological examination of specimens taken from thyroid nodules by FNAB has been one of the most significant advances in the investigation of thyroid disease in the past decade. Unfortunately, there remains the same limitations to the technique as for histological assessment, namely the inability of the fine needle cytology to accurately discriminate between benign follicular adenomas and follicular carcinomas [22]. Cytological techniques are limited to assessing cellular characteristics, and cannot determine whether or not follicular cells have actually penetrated the thyroid capsule or invaded blood vessels, the two principal histological determinants of follicular thyroid cancer. MRS on FNAB offers the prospect of a technique which more accurately reflects the actual biology of follicular thyroid tumours, allowing more accurate diagnosis of thyroid cancers and reducing the need for unnecessary surgery performed solely for diagnostic purposes. It is possible to obtain an accurate ^{1}H MR spectrum from as few as 10^{6} thyroid cells, obtained from a FNAB [3].

In addition, in a study of FNAB and tissue specimens from 70 patients undergoing thyroidectomy for solitary or dominant thyroid nodules, a close correlation between fine needle MR spectra and tissue spectra for a range of benign and malignant neoplasms has been demonstrated [3]. The sensitivity or probability of correctly identifying thyroid cancer on the basis of MRS assessed on FNAB was 95%. On the basis of this high sensitivity of the test, a clinical trial has now commenced in Australia aimed at

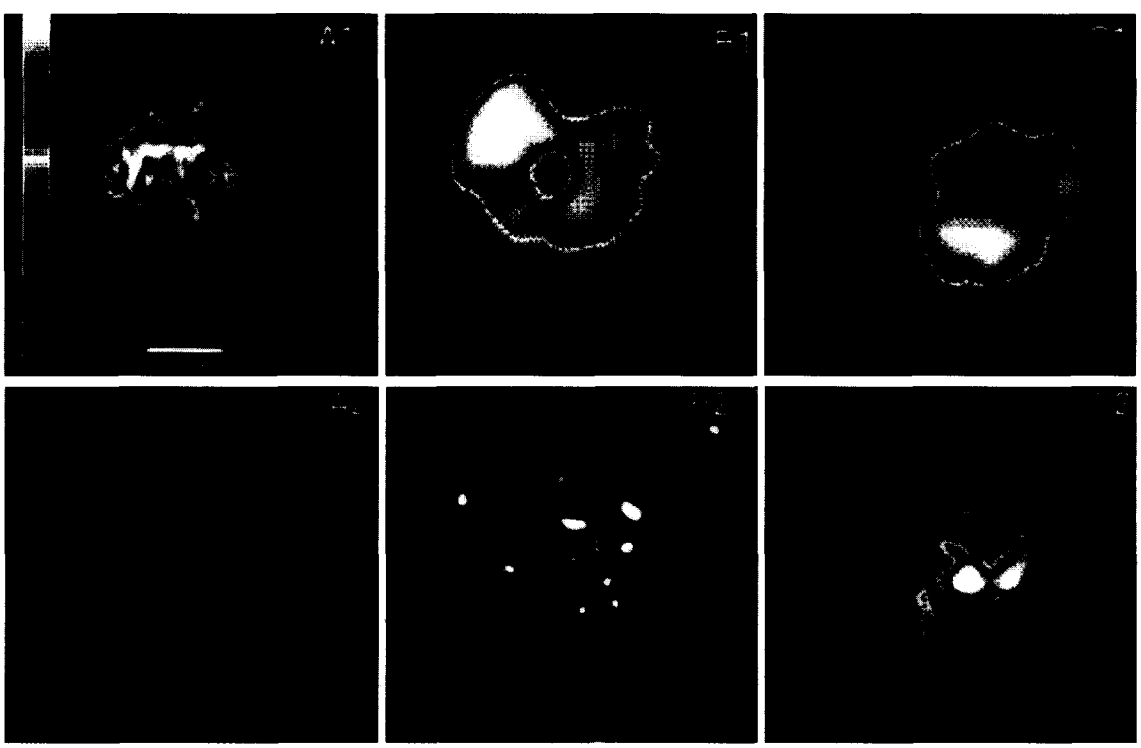

Fig. 4. MR CSI of three follicular thyroid neoplasms were obtained (ex vivo) at the frequency of water (A1, B1, C1) and the methyl moiety of lipid and amino acids (A2, B2, C2). The histological and clinical diagnoses of the tissue specimens were as follows: (A) benign follicular neoplasm; (B) benign follicular neoplasm and (C) follicular neoplasm – not histologically malignant but known to be invasive due to a secondary tumour identified in the patient. The methyl images identify changes in cellular chemistry occurring in foci during the progression of malignant disease and support the heterogenous nature of follicular thyroid tissue. A Gaussian filter was applied prior to Fourier transformation to enhance signal to noise of the methyl images.

identifying 'atypical follicular lesions' on FNAB which are genuinely benign, therefore avoiding expensive and unnecessary surgical procedures.

4.3. Chemical shift imaging

Conventional magnetic resonance imaging (MRI) can visualise gross lesions of the thyroid but is unable to determine the malignant potential of such lesions [23], although the presence of variable thickness of the capsule have been deemed correlative of benign or malignant neoplasia [24]. The inability to discriminate, using water-based MRI, is to be expected given the non-specific increase in free water content that occurs in inflammatory, hyperplastic and neoplastic diseases of the thyroid [25].

Spectroscopy is the MR technique of choice to assess follicular neoplasms and has recorded a variation in MR spectral patterns for histologically non-malignant thyroid follicular neoplasms suggesting that their cellular chemistry is heterogeneous [2,3]. Since single voxel MRS examines the entire sample it does not indicate whether or not the changes in cellular chemistry occur uniformly throughout the tissue. Evidence of chemical heterogeneity in follicular neoplasms which are morphologically homogeneous have been demonstrated using CSI ex vivo.

Fig. 4 shows water and methyl images of 3 separate follicular neoplasms, two benign adenomas (Fig. 4A and B) and a proven follicular carcinoma (Fig. 4C). The intensity of the methyl image was variable throughout the entire sample, ranging from no signal recorded (Fig. 4A), through weak scattered areas of methyl signal (Fig. 4B) in adenomas, to intense areas of signal in the carcinoma. The methyl images from benign adenoma specimens indicate that the changes in cellular chemistry are occurring in foci. These foci of altered chemistry were observed in 6 out of 10 histologically and clinically benign follicular adenomas [5].

MR images were correlated with histopathological sections in the same plane, to ascertain if any morphological features could be identified which corresponded to the location of the foci of intense methyl signal and to determine if an overall cellular morphology or tissue composition could be correlated with the general appearance of the methyl image. The appearance of the follicular cells and the presence of colloid, fibrous septa or blood vessels could not be correlated with the location of the foci of intensity in the methyl images. In all cases, there was nothing to distinguish the cellular morphology at the location of the methyl intensity from any other portion of the biopsy. Thus, features or changes in cellular morphology visible by light microscopy were not spatially correlated to the intensity of the MR methyl images. MR microimaging is apparently reporting on tissue heterogeneity which is not visible using the light microscope.

In these experiments, using an 8.5 Tesla magnet, the size of a single voxel in the images was $40 \times 40 \times 500$ μm. The diameter of a follicular cell is approximately 25 μm, therefore in the plane of the image there are 3–4 cells per voxel. The slice thickness is equivalent to the diameter of 20–25 cells. Thus, the microimaging method is identifying chemical changes taking place in foci of about 100 cells or less. CSI demonstrated that the follicular carcinomas and 60% of the histologically and clinically benign follicular neoplasms studied were chemically heterogeneous at a cellular level.

5. Computer control of data analysis

The resonances used for diagnosis in the clinical studies described above were those easily identified by eye and for which the intensity could be calculated manually and compared to the intensity of a second spectral resonance used as an internal reference. These resonances are known to correlate with specific biological features. There are, however, a plethora of other spectral differences not readily discerned by eye which may also be diagnostic of specific pathologies. For this information to be useful it is necessary to use mathematical procedures which look for similarities in the MR data within groups of different samples, and classify them after a thorough examination of all the available information.

5.1. Multivariate analysis

The application of multivariate analysis and neural net techniques has allowed further refinement of the discriminatory potential of spectral analysis. The

most common conventional method is principal component analysis (PCA) [26] which is used for preliminary characterisation of the data sets, in particular for data reduction. Optimal region selection methods are also required which have the advantage of retaining spectral information essential for subsequent biochemical classifications. Independent analyses of different regions of the spectra are then undertaken. The analyses are typically performed using several types of classifiers, including neural nets [26], linear and quadratic discriminant analysis [27], and genetic programming [28]. These methods have already shown superiority over any other approach in analysis of MRS data for cervical tumours [17]. The results of the various methods, on the several regions of the spectra, are combined in a consensus analysis, to increase significantly the classification power of the process.

For cancer of the thyroid, no individual classification method, including linear discriminant analysis, genetic programming or neural nets, was capable of high classification accuracy on its own. Therefore, a meta classifier was developed which used the outputs of the individual classifiers as inputs to another classifier. This led to a much higher accuracy than any individual classifier could provide [29].

These studies have illustrated that no one general classification method will work well on all spectroscopic data. For each new data set from a particular organ, a specific set of methods must be developed to achieve maximum discrimination. Attention must be paid to experimental details, such as phasing in the case of MRS data, preprocessing, and the degree of sophistication of the multivariate method necessary for a particular MRS data set [29].

6. The future

At present, it is clear that water-based MRI can spatially delineate a lesion but is most often unable to provide diagnostic parameters. ^{1}H MRS, on the other hand, is able to determine objectively the chemical markers of established biological and pathological criteria from excised biopsies and fine needle aspirates (FNAB). The MR method is highly sensitive and able to detect relatively small populations of abnormal cells (e.g. fine need biopsies versus solid tissue) [1,3]. It is of great significance that MRS is able to identify pre-invasive states. This is particularly valuable since recognition of pre-invasive states with inherent malignant potential by current histopathological techniques is often contentious. By the year 2000, we believe MR techniques will play a significant role in the diagnosis and management of malignant disease. In particular, a combination of CSI aided image guided biopsy and correlation of biopsy histology with in vivo localised ^{1}H MRS data will: (a) lead to improved assessment of the extent of disease and (b) establish the sensitivity and specificity of in vivo ^{1}H MRS for the simultaneous determination of the size, location and neoplastic potential of a tumour mass.

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