NEW TECHNIQUES OF IHC AND EQA

Keith Miller
Histopathology, University College London

Immunohistochemistry on routinely processed paraffin-wax sections has been through many different evolutionary cycles over recent years. Initially, overcoming the cross linking of formalin fixation appeared to be insurmountable thus leaving many antigens undetectable in paraffin sections. Trypsin digestion was first tried with some success and then along came heat mediated antigen retrieval. The latter has been far more successful, especially when a range of different pH fluids are employed1. Next came the biomarkers that impact directly on patient care. Antibodies against hormonal receptors were the first to directly influence therapeutic decisions for breast cancer and now of course we have HER-2 testing2&3. New therapies are regularly emerging and the effectiveness of some are often predicted by antigen expression. An example is imatinib (STI571)4 from Novartis for the treatment of gastrointestinal stromal cell tumours that express c-kit (CD117). The effectiveness on some patients has been quite spectacular.

With immunohistochemical tests now having more and more impact on patient care it is vital that greater standardisation of immunotechniques occurs. How this is best achieved is dependent on the philosophy towards standardisation in various regions. Some have formed discussion groups, whilst others have organised web sites with methodologies. The experience of the UK NEQAS for Immunocytochemistry indicates that some improvements are urgently required5. However, over the longer term it is likely that complete automation of the process coupled with strict EQA will provide the answers.

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MOLECULAR GENETICS OF SPORADIC AND FAMILIAL BREAST CANCER

Sunil R Lakhani
The Breakthrough Toby Robins Breast Cancer Research Centre & The Royal Marsden Hospital, London

The increasing use of mammographic screening over the last decade has led to an increased detection of preinvasive breast disease and highlighted the deficiencies in the pathological classification and our understanding of the biology of breast cancer. The development of quality assurance schemes (EQA) has led to considerable improvements in the standardisation of criteria and reducing inter-observer variability amongst pathologists. Yet, some types of proliferations within the breast, such as atypical hyperplasia, remain problematic. There is a hope that the new molecular techniques will help to provide a molecular classification that is more robust and clinically useful.

Technological advances over the last 2 decades has resulted in the development of techniques such as Loss of heterozygosity (LOH), Comparative Genomic Hybridisation (CGH), high throughput mutational analysis, DNA sequencing and microarrays. These techniques, together with the use of immunohistochemistry on tissue microarrays have made it easier to identify cancer-causing alterations, as well as changes in patterns of gene expression and protein function. Researchers are now in a position to investigate and quantify the many complex changes that occur during tumorigenesis.

The techniques have been used to study differences between sporadic and familial cancers as well as preinvasive breast disease.

One of the most significant contributions has been in delineating the pathways for the development of cancer from preinvasive breast disease. CGH analysis has contributed considerable data in recognising that low grade DCIS and low-grade invasive breast cancers arise via a separate pathway from high grade DCIS and high-grade invasive carcinoma. Hence, the traditional concept of 'de-differentiation' of cancers with time appears to be wrong. The application of array technology to CGH will markedly improve the resolution of the technique.

As well as cataloguing the amplifications, deletions and complex rearrangements at the genomic (DNA) level, analysis of changes in the profiles of gene expression (mRNA) may give clues to the underlying molecular events in breast tumorigenesis. The power of this technique has been elegantly demonstrated in a number of recent papers

Pathological assessment of tissues has remained the linchpin of diagnostic practice for over a hundred years. It has become the core science of clinical medical practice, providing data for clinical management and a framework for future correlation of new markers and new therapies. With the current explosion of technology and data, it is important for pathologists and other clinical specialists to embrace and incorporate these changes into their training and practice. Molecular biologists will also benefit from a closer interaction with pathologists.

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MICROMETASTASES IN BREAST CANCER DETECTION AND CLINICAL RELEVANCE

Nesland Jahn M.
Department of Pathology, The Norwegian Radium Hospital, Norway

An increasing number of breast cancer patients are cured, due to early diagnosis and improved primary treatment, but still 20-30% of lymph node negative and 60% of lymph node positive breast carcinoma patients will experience a systemic relapse. Adjuvant treatment (chemotherapy and/or endocrine treatment) after primary surgery gives a survival benefit of 10-30% (total and relative survival). Thus, it is possible to cure breast cancer patients in a micrometastatic stage. The questions are how to detect micrometastases and how to individualize treatment. At present TNM classification, tumor grade, hormone receptor status and age are used for prognostication and treatment decisions. However, these factors are not sufficient for an accurate selection of patients into low risk groups with no need for adjuvant treatment, and high risk groups for systemic relapse.

The metastatic process

The metastatic process involves a series of steps, such as cell proliferation, cell mobility, proteolysis, vascular invasion (hematogenous and lymphatic), survival in the circulation, cellla dhesion and finally establishing a metastatic focus in the remote place. Tumor cells may enter the circulation both by lymphatic vessels and blood vessels. The phenotypic expression in cells in blood vessels is different from what is found in tumor cells in lymphatic vessels.

Lymph node metastases

The presence of lymph node metastases is the most important prognostic factor in breast cancer patients. The introduction of sentinel lymph node biopsy (SLNB) (1991) has reduced the number of axillary lymph node dissections (ALND). However, several questions are unsolved. What is the optimal procedure? How many sections and what is the importance of single cells and small groups of cells in lymph nodes are not settled. What about the use of immunohistochemistry, which give an increase of more than 6% in positive events?

At present an injection of blue dye in the parenchyma around the tumor and injection of an isotope in the skin above the tumor is recommended. We do know that SLN negativity in H/E stained sections and with immunohistochemistry predicts no relapse (at least for the 4 years to come). The problem is how to evaluate the impact of tumor cells detected with immunohistochemistry alone. Aggregates of tumor cells larger than 2 mm are associated with an anfavourable outcome.

Micrometastases in the bone marrow

Presence of isolated tumor cells in the bone marrow at time of primary surgical intervention is shown to be a predictor of early relapse in node positive patients in several studies. The reported impact in node negative patients has been contradictory.

920 patients with breast carcinoma in the Oslo region were in the period 1995-1998 enrolled in a study of micrometastatic disease. BM aspiration was performed prior to surgery. Cytospins of mononuclear cell suspensions were immunostained for cytokeratins (AE1/AE3) and 2 mill cells were examined. A standardized protocol for evaluation was followed. At the same time a negative immunomagnetic separation technique was applied. Anti CD 45- conjugated Dynabeads were used in separation of cells. Non-rosettes cells in a magnetic field were collected and immunostained for cytokeratins.

The primary tumors were characterized and in addition to grade (Elston/Ellis), type, and size, immunoreactivity for ER alpha, PgR, p53 protein, Her2, and cathepsin D was evaluated.

Tumor size was <2cm in 61% of the patients. 63% of all patients were lymph node negative. Direct ICC detected isolated tumor cells in 13.2 %. The presence of tumor cells correlated with the nodal and tumor stage, with 10% positive events in the node negative group and 20% in the in node positive group. Presence of tumor cells in the bone marrow increased with increasing T status (11% in T1, 15% in T2 and 23% in T3-T4). An association between presence ot tumor cells in the BM and ER/PgR negativity in the primary tumors was observed. Systemic relapse and death of breast cancer occurred in 32% and 27% of the BM+ group versus 14% and 11% in the BM-group after a median follow up of 49 months. In multivariant analyses BM findings was an independent prognosticator together with established factors. In the node negative group no such impact of BM findings was observed, which could be due to the relative limited follow up period. Methodological aspects determine the impact of BM analysis when the micrometastatic load is low. Presence of false positive cells can create a problem. In our experience we can anticipate 3-4% false positive reactions, and an extensive control set up is necessary.

A predilection of distant metastases to the skeleton has previously been reported. We did observe that, in addition, that the rate of liver metastases, but not lung metastases was significantly higher in the BM+ group.

Conclusion

Detection of circulating tumor cells in the bone marrow is a promising prognosticator and predictor of treatment response. It can also be used to monitor the treatment effect. Several detection methods are applied. At present the use of immunocytochemistry with or without immunomagnetic separation and a set up with standardized controls is recommended.

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APOPTOSIS, PROLIFERATION AND BCL-2 OVEREXPRESSION IN BREAST DISEASE

Niki J. Agnantis
Department of Pathology, University of Ioannina Medical School, Ioannina, Greece

Disruption of the balance between apoptosis and proliferation is considered to be an important factor in the development and progression of tumors. An absolute requirement for a cell in order to undergo apoptosis is its progression through the cell cycle [1]. The proto-oncogene bcl-2 is involved in the regulation of normal cell cycle, inhibiting apoptosis in normal as well as neoplastic tissues. Bcl-2 started being investigated in breast cancer relatively recently. Initial studies suggested a prognostic significance of bcl-2 protein expression, although its independent value in multivariate survival analysis was questioned and by others these findings were initially considered paradoxical [1,2,4]. However, the favorable prognostic significance of bcl-2 expression in breast cancer has been demonstrated in several published series. Intense expression of bcl-2 protein was also found independently associated with a better clinical outcome of breast cancer.

In our laboratory we have determined the in vivo cell kinetics along the spectrum of apparently normal epithelium, hyperplasia, preinvasive lesion and invasive carcinoma, in breast tissues affected by fibrocystic disease in which preivasive and/or invasive lesions developed, as a model of breast carcinogenesis [4]. In addition we have determined the bcl-2 protein expression in operable breast cancer and correlated the expression of the protein with clinical outcome [5].

For assessing the aspect of apoptosis/proliferation a total of 32 areas of apparently normal epithelium and 135 ductal proliferative and neoplastic lesions were studied. More than one epithelial lesion per case were analyzed. The apoptotic index (AI) and the proliferative index (PI) were expressed as the percentage of TdT-mediated dUTP-nick end-labeling (TUNEL) and Ki-67-positive cells, respectively. The PI/AI (P/A index) was calculated for each case. We have found that the AIs and PIs were significantly higher in hyperplasia than in apparently normal epithelium ($P=0.04$ and $P=0.0005$, respectively), in atypical hyperplasia than in hypeplasia ($P=0.01$ and $P=0.04$, respectively) and in invasive carcinoma than in in situ carcinoma ($P<0.001$ and $P<0.001$, respectively). The two indices were similar in atypical hyperplasia and in in situ carcinoma. The P/A index increased significantly from normal epithelium to hypeprlasia ($P=0.01$) and from preinvasive lesions to invasive carcinoma ($P=0.04$) whereas it was decreased (non-significantly) from hyperplasia to preinvasive lesions. A strong positive correlation between the AIs and PIs was found ($r=0.83$, $P<0.001$).

Our findings suggest accelerating cell turnover along the continuum of breast carcinogenesis. Atypical hyperplasias and in situ carcinomas might be kinetically similar lesions. In the transition from normal epithelium to hyperplasia and from preinvasive lesions to invasive carcinoma the net growth of epithelial cells results from a growth imbalance in favor of proliferation. In the transition from hypeprlasia to preinvasive lesions there is an imbalance in favor of apoptosis.

Regarding bcl-2, we have examined by immunohistochemistry the expression of bcl-2 protein in sixty-eight cases of operable breast cancer (stage I, II and III) and correlated with disease outcome. The tumors were classified according to the WHO criteria. The age of the patients ranged from 26 to 76 years (median age 52 years). They had primarily been diagnosed and treated in the Ioannina University Hospital (1990-1998) and were staged according to the American Joint Committee on Cancer (AJCC) classification and stage grouping system. All patients were clinically disease-free and had baseline CA 15-3 serum levels below 30 U/mL post-surgically, at initiation of adjuvant therapy. All patients of this study had a regular follow up every 4 months during first 2 post mastectomy years and every 6 months thereafter. Immunostaining was performed on formalin-fixed, paraffin-embedded tissue sections using a streptavidin-biotin complex reagent. Antibody directed against bcl-2 protein (clone:124. Dako; dilution 1:40), were applied. The percentage of positive cells was estimated by scoring the slide as follows: 0-20% positive tumor cells = negative or weak expression, 30-90% positive tumor cells = moderate expression, >90% positive tumor cells = absolute expression.

Immunostaining for bcl-2 was negative to weak in 36.7% of the cases and involved approximately the entire neoplastic population in other 38.3%. In the rest quarter, 30-90% of the tumor cells were stained positive. In disease-free time and overall survival analysis we found an impressive positive impact of absolute overexpression of bcl-2 on clinical outcome ($P=0.001$).

We have thus demonstrated an impressively positive impact of absolute overexpression of bcl-2 on clinical outcome, both disease-free time and overall survival. No one of the patients who hosted an operable breast cancer with absolute overexpression of bcl-2, relapsed at a median/mean follow up time of 14/40 months. We consider that, notwithstanding a rather modest number of evaluated patients in this study, the strength of the obtained statistical significance provides sound evidence for clinical importance of assessment of bcl-2 at the level of absolute overexpression. Although our findings call for proper confirmation in bigger series, it should be pointed that this study provides evidence that the assessment of absolute overexpression of bcl-2 constitutes a new approach that might provide important prognostic information in operable breast cancer.

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MOLECULAR PATHOLOGY OF ENDOMETRIAL HYPERPLASIA AND CARCINOMA

Jaime Prat.
Department of Pathology, Hospital de la Santa Creu i Sant Pau, Autonomous University of Barcelona, Spain

In the Western World, endometrial cancer is the most common malignant tumor of the female genital tract. After an increase in the 70s that resulted from the unrestricted use of estrogen replacement therapy in postmenopausal women, the incidence rates became stable over the last two decades (10-20 per 100,000 person-years). Recently, the progressive use of tamoxifen - a non-steroidal estrogen agonist and antagonist - for the treatment of breast cancer has been associated with increased risk of endometrial cancer but there is not complete agreement among different studies. In this review, current knowledge about molecular pathology of endometrial carcinomas and their precursors will be presented.

TWO TYPES OF ENDOMETRIAL CARCINOMA

Over the past two decades, the tendency has been to classify endometrial carcinoma (EC)1 into two different types: **Type I** tumors (about 80%) are **endometrioid carcinomas**, often preceded by complex and atypical hyperplasia and associated with estrogenic stimulation. They occur predominantly in pre or perimenopausal women and are associated with obesity, hyperlipidemia, anovulation, infertility, and late menopause. Typically, most endometrioid carcinomas are limited to the uterus and follow a favorable course. In contrast, **type II** tumors (about 10%) are **non-endometrioid** (largely papillary serous) **carcinomas**, arising occasionally in endometrial polyps or from precancerous lesions that develop in atrophic endometria (endometrial "intraepithelial" carcinoma)2. Type II tumors are not associated with estrogen stimulation or hyperplasia, readily invade the myometrium and vascular spaces, and carry a high mortality rate. It has also been found that the molecular alterations involved in the development of endometrioid (type I) carcinomas are different from those of the non-endometrioid (type II) carcinomas3-4 (Table 1).

MOLECULAR PATHOLOGY

A dualistic model of endometrial carcinogenesis has been proposed.1-3 According to this model, normal endometrial cells would transform into endometrioid carcinomas (EEC) through replication errors, so-called "microsatellite instability" (MI), and subsequent accumulation of mutations in oncogenes and tumor supressor genes, whereas alterations of p535 and loss of heterozygosity (LOH) on several chromosomes would drive the process of neoplastic transformation into the acquisition of a non-endometrioid carcinoma (NEEC) phenotype4, 5.

There are several evidences in favor of this pathogenetic proposal; the majority of low grade EEC express estrogen receptors but not p53, and 25 to 30% of them exhibit MI. In contrast, most NEEC are negative or only weakly immunoreactive for estrogen receptors, strongly positive for p53 immunostaining, and do not exhibit MI6.

Although the dualistic model appears applicable to paradigmatic cases at both clinicopathological and molecular levels, exceptions occur. After all, there is a great overlapping in the clinical, pathological, immunohistochemical and molecular characteristics of the tumors. For instance, it has been shown that occasionally, NEEC may develop from preexisting EEC as a result of tumor progression. Obviously, these tumors may share the pathologic and molecular features of types I and II EC4.

Molecular alterations of endometrioid carcinomas of the endometrium (EEC)

Four main molecular alterations have been described in EEC: MI (25-30% of the cases)4, 7, 8, PTEN mutations (37-61%)9-14, k-RAS mutations (10-30%)15-19, and beta-catenin mutations with nuclear protein accumulation (25-38%)20. Although MI and PTEN or k-RAS mutations may coexist in many cases, these three molecular alterations are not usually associated with beta-catenin abnormalities (Fig.1).

Microsatellite instability (MI)

MI was initially noted in colorectal cancers of patients with the hereditary non-polyposis colon cancer syndrome (HNPCC), but also in some sporadic colon cancers. EC is the second most common tumor found in HNPCC patients. MI has been demonstrated in 75% of EC associated with HNPCC, but also in 25-30% of sporadic EC4, 7, 8.

MI occurs more frequently in EEC than in NEEC. To give further support to the hypothetical dualistic model of endometrial carcinogenesis, we investigated the presence of MI in 42 sporadic EC4. The results of our study supported the concept that MI is a common genetic abnormality in EC (28%), and appears to be more frequent in EEC (33%) than in NEEC (11%). However, the occasional detection of MI in NEEC, the lack of an inverse correlation between p53 and MI, and the frequent existence of tumors exhibiting mixed pathologic, immunohistochemical and molecular features of EEC and NEEC, indicate that individual tumors do not invariably follow the so-called dualistic model of endometrial carcinogenesis4.

Molecular consequences of MI in endometrioid carcinomas

It has been demonstrated that cancers showing MI, the so-called mutator phenotype, have mismatch repair deficiencies that result in the accumulations of mutations in repeated sequences in the coding mononucleotide repeats of some particular oncogenes and tumor suppressor genes. Transforming growth factor beta receptor type II (TGF-b RII), BAX, insulin-like growth factor II receptor (IGF RII), hMSH3, and hMSH6 are all putative targets of such phenomenon. These mutations are interpreted as secondary events in the mutator phenotype pathway in cancers with MI21-25.

Our results indicate that frameshift mutations occurring at coding mononucleotide repeats in these putative target genes are quite frequent in MI positive EC; mutations in one or more of these microsatellites were detected in 16 of the 24 tumors (66.6%)25. An interesting result of our study was the fact that the mutations were heterogeneously distributed in the tumors; they were found in some tumor areas but not in others. The heterogeneous distribution of the mutations suggests that they may be involved in tumor progression. The advantage of growth provided by each specific combination of mutations in a particular area of the neoplasia could lead to its overgrowth in comparison with other tumor subclones.

In a previous report, we suggested that BAX frameshift mutations could play an important role in the progression of EC with MI24. This speculation was based on the hypothesis that the presence of inactivating BAX mutations in tumors would explain the low frequency of p53 mutations in the neoplasias associated with MI, by relieving the selective pressure for p53 mutations during tumor progression. In the presence of BAX mutations, p53 mutations would not be necessary to inhibit BAX transactivation. To give further support to such hypothesis, we compared the pattern of mutations in the primary EC and their lymph node metastases25. Interestingly, in two cases BAX mutations were found in the primary EC but not in their lymph node metastases, suggesting that the tumor subclones that exhibited BAX mutations were not responsible for the dissemination of the neoplasm. In contrast, IGFIIR frameshift mutations were detected in three metastatic tumors, but only one of them also had the mutation in the corresponding primary neoplasm25. The frequent finding of these mutations in the metastatic tumors gives support to the hypothesis that IGFIIR mutations are related to tumor progression in EC with MI25.

MI is secondary to DNA altered methylation

As mentioned above, MI was initially found in colorectal carcinomas from patients with the hereditary non-polyposis colon cancer, but also in some sporadic colon cancers. In these patients, germline and somatic mutations in the MSH2 and MLH1 genes have been detected in chromosomes 2p and 3p26. However, the frequency of mismatch repair genes mutations in sporadic colonic, gastric or endometrial carcinomas with MI is very low, which suggests that other mechanisms of gene inactivation must be involved27.

It has recently been described that MLH1 promoter hypermethylation may lead to loss of MLH1 expression and subsequent development of MI in EC. We have recently detected MLH-1 promoter hypermethylation in 11 of 12 of EC with MI (91%), but in none of the MI negative tumors. On the other hand, MLH-1 promoter hypermethylation was detected in 8 of 116 (7%) cases of endometrial hyperplasia, and it was almost exclusively restricted to atypical hyperplasias with coexisting carcinomas28. These data suggest that hypermethylation of MLH-1 may be an early event in the pathogenesis of EEC, that precedes the development of MI28.

The identification of CpG island methylation in several genes (p16, TSP-1, IGF-2, HIC-1 and MLH-1) in tumors with MI suggests that altered methylation may be a preliminary alteration in the development of the microsatellite mutator phenotype.

PTEN mutations

The tumor suppressor gene designated PTEN (phosphatase and tensin homologue deleted from chromosome 10), also called MMAC1 (mutated in multiple advanced cancers) is located on chromosome 10q23.329, 30. It is reasonable to think that the genes encoding protein phosphatases, like PTEN, act as tumor suppressor genes, since their proteins may counteract the effect of the proteins encoded for the protein kinase group of protooncogenes.

LOH at chromosome 10q23 occurs in 40% of EC. Somatic PTEN mutations are also common in EC and are almost exclusively restricted to EEC, occurring in 37-61% of the cases[9-14]. Several groups of investigators have found a concordance between MI status and PTEN mutations; the mutations occur in 60-86% of MI positive EEC, but in only 24-35% of the MI negative tumors. Such results have lead to the speculation that PTEN could be a likely candidate to be target for mutations in the MI positive EC. Recently, PTEN mutations have been detected in endometrial hyperplasias with and without atypia (19% and 21% respectively), both currently regarded as precursor lesions of EEC. Moreover, identical PTEN mutations have been detected in coexisting hyperplasias and MI positive EEC, which suggests that PTEN mutations are early events in the development of EEC13, 14.

In our recent study, PTEN mutations were detected in 18 of 38 tumors (47.3%); which falls into the range of previous studies (32-55%). PTEN mutations were more frequently found in EEC (51.5%) than in NEEC (20%). Moreover, PTEN mutations were detected more commonly in MI positive tumors (66.6%), than in MI negative neoplasms (34.8%)13.

Interestingly, PTEN mutations were detected in short coding mononucleotide repeats (A)5 and (A)6 in 4 of the 10 (40%) EC with MI14. Such frameshift mutations may have the same significance than the mutations that occur in BAX , TGF-b RII, IGFIIR, MSH3, or MSH6. We could then hypothesize that PTEN (A)5 and (A)6 mutations may be secondary to deficiencies in mismatch repair that lead to the development of MI13 and so, explaning the high frequency of PTEN. In fact, mutations at the (A)5 and (A)6 short mononucleotide tracts have been previously detected in EC with MI.

k-RAS mutations

k-RAS mutations have been identified in 10-30% of EC compared to approximately 40-50% of colon carcinomas15-19. Although some authors have failed to demonstrate a correlation between k-RAS mutations and stage, grade, depth of invasion, age or clinical outcome in EC, others have described associations between k-RAS mutations and the presence of coexistant EH, lymph node metastases, and clinical outcome in postmenopausal patients above 60 years. Also, some authors have reported an almost complete absence of k-RAS mutations in papillary serous and clear cell carcinomas of the endometrium.

In our series of 58 EC, k-ras mutations occurred in 11 (18.9%) carcinomas, all of them EEC19. We found a higher frequency of k-RAS mutations in MI positive carcinomas (6/14, 42.8%) than in MI negative tumors (5/44, 11.3%), what seems to indicate that, at least in our series, k-RAS mutations are common in EC with the microsatellite mutator phenotype19.

The evidence that altered methylation in several genes occurs in MI positive carcinomas has lead to the hypothesis that MI is just a secondary alteration triggered by an abnormal hypermethylation of hMLH-1. The finding of methylation-related GCúAT transtitions in EC with MI, and their low frequency in MI- tumors, provides some basis to explain the occurrence of k-RAS mutations in MI positive EEC19.

Beta-catenin mutations

The beta-catenin gene maps to 3p21. Beta-catenin appears to be important in the functional activities of both APC and E-cadherin. Mutations in exon 3 of beta-catenin result in stabilization of the protein, cytoplasmic and nuclear accumulation, and participation in signal transduction and transcriptional activation through the formation of complexes with DNA binding proteins. Beta-catenin mutations with nuclear protein accumulation occur in 25-38% of EECs. They appear to be independent from the presence of MI20.

Summary and prospectives (Fig.2)

Putting everything together, we may hypothesize that altered methylation is an initial alteration in EEC. MLH-1 promoter hypermethylation causes mismatch repair deficiencies that produce the phenomenon of MI, and a stepwise progressive process of accumulation of mutations at coding mononucleotide repeat microsatellites in some particular oncogenes and tumor suppressor genes such as BAX, IGFIIR, MSH3, or MSH6. PTEN mutations would occur early in the process of the acquisition of the fully developed microsatellite mutator phenotype. The frequent occurrence of frameshift mutations at PTEN (A)5 and (A)6 would provide some basis to explain the close association between PTEN and MI. On the other hand, the detection of PTEN mutations in a significant percentage of MI negative EC, may also indicate that PTEN may participate in the process of endometrial carcinogenesis by following molecular pathways independent from the microsatellite mutator phenotype. The frequent occurrence of methylation-related transitions in k-RAS also provides some basis to explain the common coexistence of k-RAS mutations and MI. Finally, beta-catenin seems to play a role in EEC independently from MI.

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MOLECULAR AND CLINICAL DATA ON PIGMENTED LESIONS

Martin Charles, Mihm, Jr

In this lecture, we will discuss three aspects concerning this topic: First, genomics and personalized medicine; second, traditional markers in melanoma and finally molecular markers of melanoma. The Human Genome Project has allowed us to sequence human DNA. This human genomic sequence variation is due to single nucleotide polymorphisms, which are what make us different from one another. The technology that has developed has allowed us to compare genomics from person to person, which allows us to understand individual sensitivities in patients, that we otherwise could not fully understand previously. This information, however, must be very ethically handled and legally protected, because the type of information that, for example, insurance companies would want to probably know about patients. Thus, it is very important that appropriate legal restraints are established. In order to understand this enormous quantity of material that genomics has provided, we need a good bioinformatics and computational biology system, and training and education of appropriate personnel. These details will be discussed. We then will show the current approach to understanding prognosis by routine methods of histology and then go on to discuss in detail the molecular approach to diagnosis including routine immunochemistry, PCR evaluation, cDNA microarray analysis and proteomics.

Finally, the approach to the new staging system of melanoma as approved by the American Joint Committe on Cancer, The EORTC and The WHO melanoma committees. The comparison between the old and new systems will be discussed and clarified. Also, the significance of levels of invasion, a new method of determining lymph node metastases and the new definition of distant metastases will be reviewed.

THE IMMUNOBIOLOGY OF MELANOMA

Konstantina Frangia
Pathology Department, General Hospital of Athens "G.Gennimatas"

During the last decades, the incidence of cutaneous melanoma has been rapidly increasing all over the world, especially among Caucasians. Mortality has also been rising, although improvement in survival rate has been documented, most probably due to early diagnosis and excision of thin melanomas. Heterogeneity among individuals and various stages of tumor progression concerning both response to immunotherapy and rates of recurrence, metastasis as well as overall survival, has been also well documented. Immunopathological studies based on these observations have revealed the complexity of immune response of the host against melanoma, with the melanoma cell itself playing a significant role in the perturbation of immune surveillance. Consequently, individualized therapy not only per patient but also per stage of tumor progression, seems to be the prospective goal of medicine for a more accurate and fruitful biologic treatment of melanoma.

Today, it is well recognized that the presence of biologically 'inactive" lymphocytes in the area of a melanoma is not of biologic significance The inflammatory host response represented by strictly defined categories of tumor infiltrating lymphocytes (TILs) on the setting of a vertical growth phase melanoma, has been proved to correlate strongly with survival and according to some researchers to be a prognostic indicator independently of tumor thickness. TILs seem to play also a significant role in recurrence and evolution of nodal metastasis.

The cellular immune response to tumors requires presentation of tumor antigens to T lymphocytes. Alteration of the expression of HLA molecules encoded within the major histocompatibility locus on chromosome 6, seems to be used by melanoma to escape the immune response. Radial growth phase melanomas express HLA class I antigens, that are progressively lost during the evolution of melanoma. On the other hand, vertical growth phase and metastatic melanomas express HLA class II antigens. The expression of HLA antigens can be regulated by cytokines (IF, IL) and growth factors (TNF) which can be used therapeutically on the basis of a treatment adjusted to the stage of tumor progression. Melanoma cells express a large number of different growth factors and cytokines as well as a variety of receptors that can by used by melanomas for paracrine effects on endothelial cells, fibroblasts or macrophages (progression to metastasis through mechanism of angiogenesis)

These interactions between local cellular infiltrate (lymphocytes, dendritic cells, macrophages, NK cells etc), intracellular matrix and malignant melanocytes have been shown to represent a dynamic process that constantly alters the antigenic profile of both neoplastic cells and lymphocytes during the various steps of tumor progression. The complexity of these interactions at the present time delays the establishment of vaccine therapy or individualized treatment in melanoma patients, but has contributed greatly in recognition of melanoma associated antigens (MAA) and development of antibodies against MAAs (S100, HMB-45, NK1/C3, Mart1/ Melan A, tyrosinase etc). Many of these antibodies are currently used on diagnostic and/or therapeutic level.

Spontaneous regression of cutaneous melanoma, is a well documented entity in the literature, often associated with metastatic melanomas of unknown primary site.

The correlation of regression with survival has been controversial, due to the inconsistency of the histological criteria used to define the entity. The most commonly used and worldwide accepted definition of regression is complete absence of melanoma in the atrophic epidermis and the dilated papillary dermis, bordered in one or both sides by vertical growth phase melanoma. Dermal fibroplasia, with vascular ectasia and/or edema, as well as lymphocytic infiltrate and melanophages are characteristic features of regression. Initially, the entity had been considered as a favorable prognostic parameter due to the lymphocytic infiltrates present in the dermis. Later on studies have shown that regression is an independent predictor of survival when occurs in the radial growth phase component of melanomas that have also vertical growth phase component. More recently, thin melanomas with extensive regression have been documented to correlate with metastasis. In our experience, patients with complete regression of melanomas have been found to present with bulky lymph node or even visceral metastases, and long survival.

The immunological interaction between melanocytes, lymphocytes and the rest components of the melanoma tissue in the microenvironment of the metastatic deposit may be responsible for the regression of the tumor in the primary site.

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CHILDHOOD MELANOMA

Anna Batistatou
Department of Pathology, University of Ioannina Medical School, Ioannina, Greece

Melanoma in childhood is a rare condition, but there is evidence that it is increasing in frequency. Currently, childhood melanomas (i.e. melanomas developing before the age of 14) represent the 0.3-0.4% of all melanomas, while the percentage increases to 2% for melanomas developing in patients younger than 20 years. Melanoma accounts for 1% of all pediatric malignancies.

The risk factors for childhood melanoma include giant congenital melanocytic nevi, dysplastic nevi, xeroderma pigmentosum and immunosuppresion. The relevance of risk factors for adult melanoma, such as fair skin, blistering sunburns in young age and family history of melanoma is not clear. The incidence is the same in boys and girls. In boys melanomas are preferentially located in head, neck and trunk, while in girls in the upper and lower extremities. Children are more likely than adults to have a melanoma developing in the head and neck region.

At least one third of childhood melanomas arises within a giant congenital melanocytic nevus. The 1 in 20,000 newborns with a giant congenital nevus (greater than 20 cm in diameter in adulthood) has a lifetime risk of 4% to 7% for developing melanoma. There are two peaks of melanoma incidence: one in early childhood (first decade of life) and one in adult life. Often the melanoma arises from the deep portions of the nevus, in the reticular dermis and/or subcutaneous fat and is characterized by a highly variable morphologic appearance. It may resemble malignant blue nevus, malignant schwannoma, undifferentiated small cell neoplasm, or neoplasm from stellate cells. It is not clear yet whether small melanocytic congenital nevi (found in 1% of newborns) confer an increased risk for developing malignant melanoma. Rare melanomas arising in small or medium-sized congenital melanocytic nevi develop in the superficial component and have the same morphological appearance with melanomas of adult life.

Dysplastic nevi can occur in children, often first on the scalp. The appearance of more than 20 normal appearing nevi by age 5-6 is the first indication that a child will develop dysplastic nevi. Dysplastic nevi can be precursors of melanoma in patients with history of familial melanoma and dysplastic nevi. Familial melanomas occur most commonly in adult life, however they can appear during the teenage years.

Melanomas not arising from a preexisting pigmented lesion account for 40-50% of childhood melanomas. As in adults the most common subtype is superficial spreading melanoma. Although earlier studies have suggested a worse prognosis in children, more recent data suggest that the behavior of melanoma is similar to that in adults when major prognostic variables are corrected for.

The criteria for diagnosis of melanoma in children are the same with those in adults. In children however, the major entity included in the differential diagnosis is Spitz nevus, which is much more frequent than melanoma. Although the histologic appearance of melanoma is the same as in adults, "spitzoid-melanomas" develop more often before adolescence, making differential diagnosis difficult in young patients.

Spitz nevus is characterized by a relatively small size (usually less than 0.6 cm), symmetry, sharp lateral borders, junctional cleavage artifact, relative lack of cellular pleomorphism, evidence of maturation with increasing depth of the lesion, infrequent involvement of overlying dermis by single nevus cells (Spitz nevi developing in young children are excluded), absent cellular cohesion, and the frequent presence of PAS-positive hyaline bodies (Kamino bodies) at the dermoepidermal interface. The Spitz nevus is usually amelanotic or hypopigmented but pigmented variants are occasionally seen. Mitotic activity is usually inconspicuous, however mitoses can be observed in the epidermis or the upper one third of dermis, mostly in young children. Mitoses in Spitz nevus are not atypical. In contrast, melanoma can attain any size, is asymmetrical with "fuzzy" lateral borders, and is characterized by the presence of ascending neoplastic cells in the epidermis, the absence of maturation with depth, and the presence of large cell nests in the deep dermis. Mitoses are often conspicuous in the dermis as well as the epidermis, and are frequently atypical.

It should be pointed that, for the correct diagnosis it is important to co-evaluate the histologic findings with the clinical information (age, site, presence of pigmentation, recent change in color or diameter e.t.c.). Despite extensive investigation, some lesions defy exact classification. For such doubtful cases the close cooperation between the clinician and the pathologist is necessary for planning the appropriate therapy.

Once the diagnosis of melanoma has been established, surgical excision should be performed with relatively wide margins (1 cm for melanomas <1mm in depth and 2 cm for thicker melanomas). Sentinel node biopsy and dissection of regional lymph nodes have the same indications as in adults. Similar with melanoma in adults, there is currently no effective therapy for metastatic melanoma in children. Therefore the main focus should be risk reduction and early detection of childhood melanoma.

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MOLECULAR PATHOLOGY APPLIED TO SOLID TUMORS

Antonio Llombart-Bosch and José A. Lopez-Guerrero
Department of Pathology, Medical School, University of Valencia, Spain
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In mammals, to produce and maintain the intricate organization of the body, the component cells must obey strict controls that limit their proliferation. In adults, most cells do not grow or divide, but instead remain at rest, performing their specialized function while retired from the division cycle. However, the accumulation of genetic damage leads to the loss of several of these controls and in consequence to malignant cell transformation.

Cancer is a disease of accumulation of clonal transformed cells in which each newly acquired mutation results in an altered progeny providing a growing population of cells that leads to tumor progression. Characterization of these transformed cells constitutes the main task of the molecular pathologists of cancer.

Molecular pathology has undergone successive revolutionary changes with the introduction of new technology from the *genomic* and *proteomic* era. These technologies, such as tissue arrays, cDNA arrays or 2D gel electrophoresis, are very useful for the identification of new markers which have improved the recognition and classification of tumors.

Among the main genetic alterations observed in cancer cells are the point mutations, gene deletions, chromosomal translocations, gene amplifications and alterations in the enzymatic machinery of DNA repair. In the last decade, however, the new concept of *epigenetics* has emerged in the study of the molecular biology of the tumors. Epigenetics refers to gene modifications other than changes in the DNA sequence itself. Epigenetic modifications include the addition of molecules, like methyl groups, to the DNA backbone which acts on the gene expression. The effects of the genetic and epigenetic alterations lead to activation of oncogenes or inactivation of tumor suppressor genes which define each of the steps involved in tumor progression.

Carcinomas of the colon represent a good example where the histological adenoma-carcinoma sequence is closely associated to specific genetic changes. A normal colon acquires mutations of tumor suppressor genes such as APC or mismatch repair genes. Next, methylation abnormalities of the APC, b-catenin or MSH2 genes lead to mucosa at risk. Then, K-ras mutation or homozygous loss of additional cancer suppressor genes (such as p53 or DCC) lead to adenoma. Finally, additional mutations and gross chromosomal alterations, such as LOH of chromosome 17 and mdm2 gene amplification, lead to carcinoma and metastasis.

In soft tissue sarcomas (STS), the increased understanding of their biology is providing applications with better genetic implications, as well as a clear prognostic classification and a more adequate therapy. Recently, we have performed molecular and cytogenetic studies of accurately diagnosed soft tumors with the aim of characterizing them and finding some marker that can give us additional information regarding the biology and behavior of these neoplasms.

Rhabdomyosarcoma (RMS) constitutes the most common STS in childhood. Histologically, these tumors are subdivided into the alveolar (ARMS) and embryonal (ERMS) subtypes. All cases that we investigated showed clonal, numerical and structural chromosomal abnormalities. Two of the three ARMS had the t(2;13)(q35;q14), and the other showed a PAX7/FKHR fusion by RT-PCR. One of the ERMS showed a der(11)t(3;11)(p21;p15) as a sole structural anomaly. MYCN amplification in the form of double minutes or homogeneously staining regions was also observed in two ARMS. Hence, it seems evident that ARMS and ERMS are entities with different genetic characteristics, the characterization of which may have a diagnostic value as well as prognostic significance.

Chondrosarcoma (CHS) are malignant cartilage-forming tumors which represent the second most common malignant tumor of bone. We established and characterized a new human chrondrosarcoma cell line (ch-2879) from a recurrent grade III CHS of the chest wall. This cell line expresses several immunohistochemical markers, and cytogenetically, is hypotriploid displaying a cryptic 9p21 deletion. Establishment of cell lines from these poorly understood bone tumors is crucial for a better understaining, by means of *in vivo* and *in vitro* studies, of the biology and perspectives for treatment of these STS.

Human osteosarcoma (OS) is the most common form of malignant bone tumor. OS displays complex karyotypes with numerical changes as well as structural abnormalities. We found that cases with molecular alterations of the G_1 to S-phase checkpoint genes died during their clinical follow-up, whereas more than 53% of the remaining cases survived during this period. Cytogenetically, all cases had complex karyotypes, the losses of the chromosomes 4, 13 and 17 being especially significant. Thus, in the pathogenesis of human OS, deregulation of the G1/S checkpoint genes plays an important role and defines a subgroup of patients with a poor outcome.

One of the STS in which molecular studies have increased over the last five years is the gastrointestinal stromal tumor (GIST). Virtually all GISTs, display strong immunostaining for the KIT receptor. It has been described that KIT mutations result in constitutive activation of the tyrosine kinase activity of the KIT protein. The development of new drugs against tyrosine kinase activities, such as STI-571, enables an attack on a specific molecular target in GISTs. Hence, GISTs now serve as the model of solid tumors for a molecular biology-based diagnosis and treatment of cancer.

Recent advances in our understanding of the cell cycle machinery in urothelial bladder neoplasms (UBN), have demonstrated that disruption of normal G1 to S-phase cell cycle transition is one of the most frequent alterations in these tumors. We have found that LOH of 9p21 is associated with low grade and stage neoplasms, whereas amplification of MDM2 and cyclin D1 are present in advanced stage tumors. These studies emphasize the implication of multiple G1 to S-phase cell cycle regulators in the natural history of UBN. If such patients could be identified at presentation, more aggressive treatment might result in a cure.

In conclusion, as a consequence of the latest advances in genetic and proteomic technologies, and hence, in the knowledge of the molecular mechanisms of solid tumors, modern pathologists need to know the technology applicable to diagnosis regarding tissues, cells and organic fluids in molecular biology as well as the bases of molecular biology and their application to diagnosis.

MOLECULAR PATHOLOGY OF PROSTATIC PRECURSOR LESIONS

Paul H. Duray, Bethesda

Proving the link between PIN and Prostate Cancer uses many technologises: microsatellite-based loss of heterozygosity (LOH) analysis, fluorescence in situ hybridization (FISH), comparative genome hybridization (CGH), DNA methylation, multiple types of microarrays (DNA, mRNA, oligonucleotides, SiRNA) for gene expression libraries and expression profiles, and proteomics. The shared allelic loss patterns were the first genetic evidence of a link between PIN and carcinoma. LOH studies in our group revealed a deletion frequency of 63% on chromosome band 8p21 compared with 80% in carcinoma samples from the same group of patients 8q is the most frequent genetic anomaly in both PIN and carcinoma. Is this related to amplification of the c-myc gene located on chromosome band 8q24? Additional molecular lesions in Prostate cancer already present in PIN are: LOH at 8p22, 12pter-p12, 10q11.2, and GAINS in chromosomes 7,8,10 and 12. Bcl2 expression and RER+ phenotype are very much the same in PCA and in PIN. Hypermethylation of CPG islands are operative in many cancers and this has been shown with parallel loss of expression of GSTP1 in prostate cancer and PIN.

PIN foci develop independently and multifocally within the peripheral zone of the prostate gland approximately five to ten years before the onset of cancer. Could the link beinactivation of a tumor suppression gene located at chromosome band 8p21, methylation of the GSTP1 gene promoter and loss of expression, and activation of a gene located on chromosomal arm 8q, possibly c-myc? Subsequent mutations and/or genomic alterations in genes located on chromosomal arms 7q, 13q, and chromosomal band 8p22 could thenresult in progression of PIN to invasive tumors. Gene expression levels of a number of cell regulatory genes, growth factors, and prostate-specific markers has been examined in PIN and compared with benign prostate epithelium and prostate carcinoma. But expression of such genes is intermediate in PIN, and furthermore, overexpression of p53 is infrequent in organ-confined prostate carcinoma, but is completely absent in high grade PIN. However, interestingly, bcl-2 levels are significantly higher in PIN than in either carcinoma or benign prostate tissue. Bax is expressed in all carcinoma and PIN samples. Telomerase activity has been detected in a low percentage of PIN foci (16%) compared to carcinomas (69%). c-Met expression is similar in PIN (36%) and latent cancer (33%), but is much more common in clinical disease (81%) and is found in 100% of metastatic lesions. In a study examining expression of p27KIP1, the labeling index decreased incrementally from normal tissue (86.4%) through PIN (59.3%) and primary carcinoma (43.5%) to lymph node metastases (7%) (Erdamar, 1999). There is a trend toward higher expression of membranous EGFR and c-erbB2 and cytoplasmic TGF-alpha, and lower expression of FGF-2 in high grade PIN and carcinoma than in low grade PIN or BPH as determined by immunohistochemistry. Taken together, these gene expression results are consistent with the hypothesis that HGPIN is a premalignant lesion.

Our group at the NCI has also observed qualitative changes in gene expression in PIN secondary to alterations in transcript splicing. For example, we detected a novel variant of PB39 transcript in a cDNA library derived from PIN cells. PB39 mRNA was previously reported as overexpressed in prostate cancer, but was not known to exist in an alternative splice form . Interestingly, based on a search of all cDNA libraries and sequences in dbEST, the novel splice variant is primarily expressed in fetal tissues and tumors and thus may be associated with the loss of cellular differentiation that occurs during prostate tumor progression. Thus, the protein product of the alternative splice form could potentially serve as a serum marker of early prostate cancer development. Another approach has been developed in our Pathology Dept. at NCI using a novel analysis to detect an immobilized protein sample, then probed with active phosphorylated signal peptides known to be operative in PCA. It was shown that PIN and PCA both showed disease progression from normal prostate to PIN to PCA in patient-matched samples by the association of increased phosphorylation of Akt suppression of apoptosis, also supported by detecting decreased phosphorylation of ERK. Also striking was the finding of an increase in phosphorylated Akt with simultaneous suppression of downstream apoptosis pathways from histologically normal prostate cells to PIN, then into invfiltraing PCA using this reverse phase protein microarray.

High Throughput Gene Expression Analysis of PIN

We tested the feasibility of performing high-throughput gene expression analysis of prostate tumor progression using expressed tag sequencing (EST) of cDNA libraries as a gene expression platform Twelve microdissection-based cDNA libraries were produced from radical prostatectomy specimens from five patients and included normal epithelium, PIN, and cancer . A total of 29,183 successful sequences were generated. Unigene clusting algorithms of sequence derived from the microdissected libraries generated 6567 different epithelial genes

We examined the expression levels in normal epithelium, PIN and cancer using a variety of statistical tests and found that a subset of ribosomal protein genes were overexpressed in the cancer libraries. This finding is expected in tumor cells presumably because of the increased requirement for protein synthesis to support cell division . Interestingly, though, these ribosomal protein mRNAs were not increased in libraries from PIN cells. This finding is at odds with most current thinking that presumes PIN develops due to a marked increase in growth rate. However, based on the present gene expression data set one can propose an alternative hypothesis. A decreased rate of cell death as opposed to an increase in cell division mediates the development of PIN, and this premise would fit the findings of decreased signaling or signal responses in the apoptosis-programmed cell death found in our reverse phase protein microarray mentioned above. In this scenario, a decreased rate of apoptosis would be an important early event in prostate tumor progression. Certainly, this hypothesis is based on a preliminary data analysis and requires testing in follow-up studies, but it illustrates the potential of global gene expression studies to generate new insights into the fundamental mechanisms that underlie the formation of PIN.

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ORIGINS OF PROSTATE CANCER, WITH SPECIAL REFERENCE TO THE PRENEOPLASTIC LESIONS.

Constantina Petraki
Dept of Pathology, Evangelismos Hospital, Athens, Greece

Origin of prostatic epithelium

In adult men, prostatic epithelium consists of two histologically distinct compartments: a luminal and a basal. The luminal compartment consists of a luminal layer of androgen-dependent columnar secretory cells that express secretory proteins (PSA, other kallikreins and PSAP), CD 57, androgen receptors (AR), and cytokeratins 8 and 18 (CK8, CK18). The basal compartment that is surrounded by a basement membrane, forms a continuous layer and contains several subpopulations. The predominant basal epithelial subpopulation expresses CK5, CK14, CK15, CD44, p63, pp32, low levels of AR (although this is controversial), c-erbB-2, EGFR, telomerase, Ki-67, glutathione-S-transferase-pi (GST-pi) and bcl2. Based on prostate research studies, as well as on the studies of stem cells in other tissues, several researchers have proposed the differentiation of the prostatic epithelium from common progenitor/stem cells, located in the embryonic urogenital sinus epithelium, and the existence of a stem cell compartment within this epithelium (***1, 2***). This is hypothesized to correspond to a small subpopulation of androgen-independent basal cells that exhibit a cell differentiation marker profile similar to that expressed in embryonic urogenital sinus epithelium, co-expressing luminal cytokeratins (CK8, CK18), the basal cell cytokeratins (CK14, CK5), p63, pp32, telomerase, and the so-called transitional or intermediate cell markers, cytokeratin 19 and GSTpi. These stem cells may give rise to an amplifying or transiently proliferating compartment, which are pluri-potential more rapidly cycling androgen independent but androgen-responsive cells that in turn generate basal cells, differentiated luminal cells, and possibly the neuroendocrine cells, which are androgen-independent postmitotic cells dispersed throughout the basal layer. In contrast to the reserve stem cells, transiently proliferating (TP) cells, although actively cycling, have a low capacity for self renewal ***(1)***.

Origin of prostate cancer – Stem cell model

The understanding of the origins of prostate carcinoma (PCa) is mainly based on the study of the lineage relationships within the prostatic epithelium. Although basal cells are totally absent in PCa, basal cell markers are often expressed in combination with luminal cell markers by the tumor. One possible explanation is that tumor cells arise from the TP cells, and thus may have characteristics of both the luminal and basal phenotype. These TP cells could represent a subset located within the secretory epithelium that has not yet undergone terminal differentiation. Reversion of luminal cells to a phenotypically less differentiated state during the carcinogenesis could be another explanation. (***1, 2, 6***).

Preneoplastic lesions of the prostate

Putative preneoplastic lesions in the prostate gland have been studied for over 70 years. Based mainly on morphological criteria, the only putative precursor for PCa is prostatic intraepithelial neoplasia (PIN). Atypical adenomatous hyperplasia (AAH), malignancy-associated changes (MAC), and atrophy might be premalignant, but as there is no convincing data, they are probably conditions associated with a significantly increased risk of cancer (***1, 3-5***).

A. Prostatic intraepithelial neoplasia

McNeal first described PIN as an entity in the 1960s. In the 1980s, it was more precisely characterized, called intraductal dysplasia and subcategorized into three grades. In 1987, Bostwick and Brawer proposed the term PIN that referred to the preinvasive end of a morphologic continuum of cellular proliferations within prostatic ducts, ductules, and acini. In 1989 compression of the PIN classification into two grades was recommended: low-grade (LG-PIN1) and high-grade (HG-PIN2 and PIN3). PIN consists of architecturally benign prostatic acini or ducts lined by cells with a spectrum of atypical cytological features, ranging from subtle changes to those that are indistinguishable from carcinoma cells. An intact or a disrupted basal cell layer always surrounds this lesion. The distinction between LG and HG PIN is based on cytological characteristics and mainly on the presence of prominent nucleoli in HG PIN. HG PIN is often accompanied by intraductal architectural disturbance. The 'maturation phenomenon' in HG PIN refers to a more benign cytological appearance of the nuclei towards the center of the lesion. With further epithelial hyperplasia, more complex architectural patterns appear such as Roman bridge and cribriform formation. The diagnosis of HG PIN may be based on marked polymorphism, prominent nuclear enlargement, and hyperchromasia. Various patterns of PIN have been recognized: flat, tufting, micropapillary, and cribriform. The tufting pattern is the most common, present in 97% of cases, although most cases have multiple patterns. Unusual subtypes of HG PIN include PIN with signet-ring features, small cell neuroendocrine PIN, PIN with mucinous features, foamy PIN, and PIN with inverted nuclei (***4, 5***).

In cases with a few small atypical glands adjacent to HG PIN, the problem is whether these glands represent outpouchings of the adjacent PIN gland or represent microinvasive cancer. Immunohistochemical stains with antibodies to high molecular weight keratin usually do not help, and repeat biopsy should be recommended (***4, 5***).

I. Relation of HG PIN to PCa

Clinical, morphological, immunohistochemical, morphometric, molecular, and genetic studies support the relation of HG PIN to PCa. Several studies provide evidence of the continuum from early PIN to early invasive cancer and indicate that HG PIN is related more closely to PCa than to benign epithelium. Although HG PIN appears to be a precursor to most types of PCa, it should not be termed 'carcinoma in situ,' as the evidence of the natural history that we have for premalignant lesions in other organs, is lacking for the prostate (***1, 3-5***).

- **Age**

Several researchers have reported the increasing frequency of HG PIN with advancing age and its association with PCa. Clinical studies suggest that PIN precedes carcinoma by ten years or more. Although PIN can be found in the third decade of life and is quite common in the sixth, clinically detectable prostate cancer is not generally obvious till the age of 60 or 70.

- **Location**

HG PIN and PCa are located chiefly in the peripheral zone of the prostate. It has been shown that HG PIN is more closely related to peripheral intermediate or HG cancers, as opposed to transition zone LG cancers. The first ones have identical cytological features with HG PIN. Cancer and PIN are frequently multicentric in the peripheral zone, indicating a 'field' effect similar to the multicentricity of urothelial carcinoma of the urinary bladder.

- **PIN in the presence of cancer**

There is an increased frequency, extent and severity of PIN in the presence of cancer. PIN coexists with cancer in >85% of cases. Also, with increasing amounts of HG PIN there are a greater number of multifocal carcinomas. PIN on biopsy has high predictive value for cancer on subsequent biopsy.

- **Histology**

HG PIN and cancer have similar architectural and cytological features. The finding of areas of HG PIN with adjacent microinvasive carcinoma is a further histological evidence that HG PIN is a preneoplastic lesion.

- **Immunophenotype – In relation to PCa origin**

HG PIN and PCa have a similar phenotype. PIN is composed of cells with a mainly luminal phenotype and undergoes regressive changes under androgen deprivation. For some biomarkers there is progressive loss of expression with increasing grades of PIN and cancer, including secretory proteins (PSA, other hKs and PSAP), CD57, p27, p63, GST-pi, and cytoskeletal proteins (keratins and vimentin). For some biomarkers there is progressive increase in expression with increasing grades of PIN and cancer, including type IV collagenase, VEGF, TGF-alpha, FGF-8, TGFβ1, iNOS, EGF, EGFR, Lewis Y antigen, c-erbB-2, telomerase, bcl2, and c-myc. Thus it is suggested that in the development of HG PIN there is a defect in the cell cycle and the basal cell layer loses its proliferative function that is transferred to secretory luminal cell types. These proliferative abnormalities are accompanied by severe disorders of the programmed cell death within the prostatic epithelium. Abnormal growth in the prostate usually results in BPH in the transition zone and cancer in the peripheral zone, indicating different target cells responding to similar stimuli. That most prostate adenocarcinomas and HG PIN did not express p63 suggests that p63 may protect prostatic epithelial cells against neoplastic transformation, perhaps through a tumor suppressor function. Since basal cells, but not secretory cells, almost uniformly express p63, it also supports that basal cells are not direct targets for neoplastic transformation. Moreover, it strengthens the hypothesis that basal cells are protected from undergoing neoplastic transformation, at least in part, by GST-pi (***7***). Yang et al recently showed as well that occasional tumor cells in metastatic PCa stain positive for 34βE12 {anti-CK1, CK5, CK10, CK14}. This finding was very rare, representing <2% of cases and <0.2% of cells, reflecting an abnormal expression for genes that are normally not expressed (***8***). All the above findings support the existence of a potential transiently proliferating subcompartment within the basal cell compartment of the human prostate (stem cell model of PCa origin).

Cell surface markers that can discriminate among specific cell lines within the prostatic epithelium may serve as targets of therapy. Recently, prostate stem cell antigen (PSCA) has been identified. Although human PSCA RNA is largely restricted to the basal cells of the prostatic epithelium, the protein is immunohistochemically detected in both basal and secretory cells. This protein may be produced during differentiation of basal to secretory cells or may be made in the basal cells and then transferred to the secretory cells. As the encoded protein is expressed on the cell surface, PSCA may be a useful marker in the study of putative prostate stem cells and in the diagnosis, prognosis, and treatment of PCa. Expression of PSCA has been observed in BPH, PIN and in PCa. Although PSCA is not specific for prostate, as it is expressed in normal basal cells and is up-regulated in PCa, it may help resolve the lineage relationships among prostatic epithelial cells and their relationship to PCa (***9***).

- **Cell proliferation and apoptosis**

Cell proliferation and apoptosis are greater in HG PIN and PCa than in the normal prostatic epithelium.

- **Basal cell layer**

The basal cell layer is intact or fragmented in HG PIN and absent in PCa.

- **Microvessel density**

Neovascularization is greater in PIN and PCa than in the normal prostate gland with a progressive increase from PIN to cancer.

- **Morphometry**

HG PIN and PCa are morphometrically similar. They have similar nuclear area, chromatin content and distribution, nuclear perimeter, nuclear diameter, nuclear roundness, as well as nucleolar number, size, and location (***10***).

- **Genetics**

HG PIN and PCa have common genetic alterations, the most common of which are: gain, deletion, and translocation of 7q22-q31; loss of 8p and gain of 8q; and loss of 10q, 16q, and 18q. Inactivation of tumor suppressor genes or overexpression of oncogenes in these regions may be important in the initiation and progression of prostate neoplasia. This strongly supports the hypothesis that PIN is the most likely precursor of PCa (***1***).

- **DNA content**

HG PIN and cancer have similar frequency of aneuploidy.

II. Differential diagnosis of PIN

PIN must be distinguished from several benign entities and from variants of PCa (***3-5***).

- **Normal anatomic structures**

Seminal vesicles and ejaculatory ducts, Cowper's glands, Paraganglionic tissue, Mesonephric remnants, Ectopic prostatic tissue of the urethra, Central zone histology

- **Hyperplasia and other benign lesions**

Benign epithelial hyperplasia, Clear cell cribriform hyperplasia

Basal cell hyperplasia (BCH)

BCH may be misdiagnosed as HG PIN as it may show cribriform pattern, prominent nucleoli and mitotic activity. In BCH there is a proliferation of small round crowded glands, whereas in PIN the glands are larger and separated from each other by a greater amount of stroma. The nuclei in BCH tend to be round and may form small solid basaloid nests. In contrast, the nuclei in PIN tend to be columnar and do not occlude the glandular lumina. In BCH, the atypical basal cells that undermine the overlying secretory cells, mostly show a parallel to the basement membrane streaming pattern. In PIN there is full thickness cytological atypia and the nuclei are oriented perpendicular to the basement membrane. The non-hyperplastic basal cells in benign glands can also have prominent nucleoli and be misdiagnosed as HG PIN. In contrast to PIN, cytokeratin 34βE12 in BCH shows multilayered staining of the basal cells.

Postatrophic hyperplasia, Simple lobular atrophy, Sclerosing adenosis, Metaplasia and reactive changes, Urothelial metaplasia, Infarction-induced atypia, Inflammation-induced atypia, Radiation-induced atypia, Nephrogenic metaplasia of the prostatic urethra

- **Carcinoma**

Acinar adenocarcinoma, Urothelial dysplasia and carcinoma

Cribriform pattern of prostatic adenocarcinoma

When atypical cribriform glands are not accompanied by small atypical infiltrating glands, the distinction between PIN and cribriform acinar adenocarcinoma is usually important. Immunohistochemical stain with 34βE12 can help in difficult cases. In areas of numerous atypical cribriform glands, a negative reaction in all the glands is diagnostic of carcinoma; positive staining, even if patchy, characterizes the lesion as cribriform PIN. A few small unstained cribriform glands on needle biopsy are not diagnostic of PCa, as even benign glands may occasionally not be labeled. Instead the diagnosis is 'Focus of atypical cribriform glands' with a comment that 'The distinction between cribriform PIN and cribriform carcinoma can not be made with certainty, and repeat biopsy is recommended'.

Ductal adenocarcinoma (DA)

Their distinction is very important, as DAs are aggressive tumors, often of an advanced pathological stage. HG PIN usually contains micropapillary projections without fibrovascular cores, while DA is characterized by true papillary formations with fibrovascular cores. PIN reveals only focal necrosis rarely, while DA may have extensive comedonecrosis. In contrast to PIN, DA may consist of very large and/or back-to-back glands. The use of high molecular weight keratin is of limited help, as both lesions may have a disrupted basal cell layer. However, absence of a basal cell layer in numerous glands rules out PIN.

Intraductal carcinoma (IDC)

In recent years, some authors have proposed that certain cribriform patterns of HG PIN should be considered as 'IDC'. IDC usually arises within established invasive cancer and represents the spread of a cancer within preexisting ducts. In contrast to PIN that is considered a premalignant lesion, IDC is a late event in tumor progression. Both lesions maintain a patchy basal cell layer. The 'maturation phenomenon,' that is considered characteristic of HG PIN, is mostly lacking in IDC. The presence of adjacent typical infiltrating acinar carcinoma favors the diagnosis of IDC. Some pathologists prefer to classify all lesions as HG PIN. However, the presence of comedonecrosis should lead to the diagnosis of IDC. Atypical cribriform glands on biopsy without necrosis, could be characterized as HG PIN, yet it should be mentioned that these lesions are more frequently associated with cancers than other forms of PIN or more correctly that these cannot be distinguished from the spread of carcinoma within ducts. In any case a repeat biopsy should strongly be recommended.

IIIa. Significance of HG PIN in needle biopsy specimens

LG PIN should not be documented as a finding in pathology reports for several reasons. It is worth mentioning that the risk of cancer with LG PIN on biopsy is the same as with benign tissue on biopsy (5-24%-average 18%) (***4, 5, 11***).

The median incidence of HG PIN in needle biopsy specimens is approximately 5% to 6% (0.7-24%), interobserver variability being the most possible reason for the observed variation. The importance of recognizing PIN on needle biopsy is its association with PCa on repeat biopsy. This risk of carcinoma on subsequent biopsy ranges from 16.5 % to 79%. The largest studies report a 23% to 35% risk of cancer on subsequent biopsy and show that if cancer is not found on the first two follow-up biopsies, it will most probably not be found. The micropapillary/cribriform pattern of PIN and a high number of involved cores are associated with a higher risk of cancer on repeat biopsy. HG PIN may appear indistinguishable from cancer as a hypoechoic lesion in ultrasound. An elevated serum PSA level in a man with HG PIN on biopsy probably reflects an unsampled cancer. Repeat prostate needle biopsy of men with HG PIN should include random sextant biopsies of the prostate, as its finding indicates a higher probability of carcinoma being found anywhere in the prostate. Identification of PIN in a prostate biopsy should not influence or dictate therapeutic decisions other than follow-up or chemoprevention. Age, general physical condition, and the preferences of the patient are taken into account for the further investigation of PIN. Although follow-up with repeat biopsy is generally recommended at 3-or 6-month intervals for 2 years and thereafter at 12-month intervals for life, there are no reasons for delaying repeat biopsy in men with HG PIN on initial biopsy. Androgen deprivation decreases the prevalence and extent of PIN, suggesting that this form of treatment may play a role in chemoprevention.

IIIb. Significance of HG PIN on TURP material

The significance of finding HG PIN on TURP is more controversial. In a younger man with HG PIN on TURP, needle biopsies should be performed to rule out a peripheral zone cancer. In an older man without elevated serum PSA levels, clinical follow-up is probably sufficient (***4, 5***).

B. Other proposed precancerous lesions

B1. Atypical adenomatous hyperplasia

AAH resembles well-differentiated adenocarcinoma architecturally, but retains an intact or discontinuous basal cell layer and lacks nuclear atypia and prominent nucleoli. It is characterized by closely packed small glands that tend to merge with the surrounding glands. Its incidence in prostate specimens varies from 2.2-23%. AAH is mostly located in the transition zone and if it is a premalignant lesion, it is probably associated with transition zone PCa. AAH has been considered a premalignant lesion on the basis of several findings, including an increased association with carcinoma, topographic relation with well-differentiated PCa, and rare cases of genetic abnormalities. But as AAH lacks the typical premalignant proliferative disorders of PIN, its role as a precursor cannot be strongly supported. Some researchers believe that the link between cancer and AAH is an epiphenomenon and that a direct transition from AAH to cancer, as observed between PIN and cancer, has not been documented (***3-5***).

B2. Malignancy-associated changes

MAC refer to abnormalities in epithelial cells that are not usually distinguishable by routine light microscopic examination. PIN and PCa are associated with or preceded by changes in the genetic material of secretory cell nuclei. The subtle morphological and molecular changes of normal looking epithelium might be seen as the onset of the development of prostatic neoplasia. Benign prostatic epithelium may show some genetic abnormalities in GST-pi and telomerase, similar to those in cancer. Changes also occur in the neovascularization of the stroma (***4, 5, 10***).

B3. Proliferative inflammatory atrophy

Atrophy and postatrophic hyperplasia of the prostate, are often associated with inflammation. Unlike in hormonal atrophy, the epithelial cells in simple atrophy/postatrophic hyperplasia have a low apoptotic and a high proliferative index. The term 'proliferative inflammatory atrophy' (PIA) has been recently introduced for these lesions. It has been also suggested that PIA may be a new candidate PCa precursor lesion, inducing a new model of prostatic carcinogenesis, in which prostatic cells in PIA lesions induce GST-pi expression as a defense against oxidative genome damage. Cells of PIA with defective GST-pi genes become sensitive to oxidants and electrophiles that cause genome damage that leads to PIN and PCa. Additionally, PIA has decreased p27Kip1, which may relate to increased proliferation and elevated levels of bcl2, which may be responsible for the low levels of cell death in this lesion. Basal cells in PIA express p63, but these are sparsely distributed relative to the basal cells in the normal glands. Luminal cells in PIA are, in general, negative for p63. These findings further support the hypothesis that intermediately differentiated luminal cells in PIA are the targets of neoplastic transformation in the prostate. Morphological transitions between HG PIN and PIA occur frequently and support a model in which the proliferative epithelium in PIA may progress to PIN and/or adenocarcinoma (***4, 5, 12, 13***).

Conclusions

In conclusion, it is strongly suggested that a stem cell, or more probably a TP cell, is the target of prostatic carcinogenesis. Prominent genetic heterogeneity and multifocality are characteristic of both HG PIN and carcinoma, suggesting a field effect in prostatic neoplasia. The strong genetic similarities between HG PIN and cancer strongly indicate that development and clonal expansion of PIN, or other precursor lesions, may account for the multifocal etiology of carcinoma.

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TRADITIONAL AND NEWER PREDICTIVE FACTORS IN PROSTATE BIOPSY WITH CANCER

Rodolfo Montironi
Institute of Pathological Anatomy and Histopathology, University of Ancona, Ancona, Italy

The pathology report of a biopsy with cancer should include a number of morphological features that may help urologists to assess the risk of extraprostatic disease and progression: cancer location, extent and volume of cancer in each specimen, and Gleason score; optional features that may add predictive value include the presence of perineural invasion, vascular/lymphatic invasion, microvessel density, and DNA ploidy expression.

Cancer grade

In prostate cancer, grade is one of the strongest predictors of biologic behavior, including invasiveness and metastatic potential. It is not sufficiently reliable when used alone, however, for predicting pathologic stage or patient outcome for individual patients. The subjective nature of grading precludes absolute precision, no matter how carefully the system is defined. Yet, the significant correlation of prostate cancer grade with virtually every outcome measure attests to the predictive strength and utility of grading in the hands of most investigators.

The Gleason system is recommended as the international standard for grading prostate cancer. The Gleason score is a scalar measurement that combines discrete primary and secondary groups (patterns or grades) into nine groups (scores 2 to 10). Gleason score should be reported as the composite score and its component patterns, e.g., Gleason 7 = 4 + 3. It is recommended that the first reported pattern is the most frequent and the second reported pattern is the second most frequent. The highest-grade pattern should also be reported, regardless of frequency. If Gleason scores must be compressed for reporting purposes, the groups should be 2-5, 6, 7, and 8-10, or 2-6, 7, and 8-10. *A global Gleason score should be given for the entirity of multiple biopsies containing cancer (in addition to individual specimen grading).*

The WHO nuclear grading system should be used in addition to the Gleason scheme, but, in practice, is rarely or never reported. Nuclear anaplasia is cored as 1, 2, and 3 corresponding to slight, moderate, and marked nuclear changes, respectively. Nuclear grade is based on the predominant nuclear grade, but there may also be value to report the highest nuclear grade.

Location of carcinoma

Transrectal ultrasound guidance allows precise anatomic sampling of the prostate, including sites other than the peripheral zone. Controversy exists regarding the utility of transition zone and seminal vesicle biopsies, but these are useful for select patients.

Routine transition zone biopsy has a low yield of cancer, ranging from 0.6% to 1.0%, with differences resulting from different patient selection criteria. Accordingly, transition zone biopsies are discouraged except in patients in whom negative sextant biopsies fail to reveal cancer but whose PSA level is markedly elevated or rising.

Seminal vesicle biopsies are most valuable in patients with PSA level more than 15 ng/ml to 20 ng/ml or abnormal or enlarged seminal vesicles according to clinical or ultrasonographic findings. For some urologists, routine seminal vesicle biopsy is advocated because positive results have a significant impact on patient management and may thus warrant additional therapy. The yield of seminal vesicle biopsy is about 15% in patients with biopsy-proven clinically localized prostate cancer and 69% in patients with metastases.

The presence of cancer within adipose tissue in needle biopsy specimens shows extraprostatic extension of cancer, which should be documented by the pathologist.

Volume of cancer

The volume of cancer in a biopsy specimen depends on many factors, including prostate volume, cancer volume, cancer distribution, technical procedure, number of biopsy cores obtained, and cohort of patients being evaluated. The combined results of several studies show that the biopsy extent of tumor has some predictive value for the extent in radical prostatectomy specimens and probably should be reported. Its predictive value for the individual patient is limited, however. Reliance on this measure alone often may be misleading. Consequently, the volume of cancer in the needle biopsy should not influence therapeutic decisions.

There is a fair to good correlation between amount of cancer reported in the biopsies and that subsequently found in radical prostatectomy specimens. This correlation is greatest for large cancers. High cancer burden on needle biopsy is strongly suggestive of large-volume, high-stage cancer. In one study, lymph node metastases were identified in 52 of 57 patients (91% specificity) and 10 of 14 patients (71% sensitivity) when adenocarcinoma completely replaced two cores and 80% of a third core from the sextant sample.

Unfortunately, low tumor burden on needle biopsy does not necessarily indicate low-volume, low-stage cancer. It was found that patients with less than 30% of needle cores replaced by cancer had mean volumes in the radical prostatectomy of 6.1 cc (range, 0.19 cc to 16.8 cc), showing that the amount of tumor on transrectal needle biopsy was not a good predictor of tumor volume. In another report, patients with less than 10% cancer in the biopsy had a 30% risk of positive surgical margins, a 27% risk of extraprostatic extension, and a 22% risk of PSA biochemical progression; these risks were higher in patients with more than 10% cancer. Patients with less than 3 mm of cancer and Gleason scores 6 or less on needle biopsy had a 59% risk of cancer volume exceeding 0.5 cc. Those with less than 2 mm of cancer had a 26% risk of extraprostatic cancer, and those with less than 3 mm had 52% risk.

The cancer volume can be measured in four different ways: (1) percentage of biopsy cores involved; (2) percentage of cancer area in each biopsy specimen; (3) millimeters of adenocarcinoma in the entire biopsy; and (4) millimeters of adenocarcinoma per core. All measures are essentially equivalent in their predictive value for cancer volume in the prostatectomy. Consequently, *the percentage of cancer area (e.g., the percentage of tissue involved by cancer) is the recommended way to measure cancer volume because it is easy to apply and will probably be accepted by most pathologists, similar to the method for measuring cancer volume in transurethral resection specimens. The pathologist should also include in the report the total percent of cancer in the total number of needle biopsy segments.* The total length of each biopsy has to be measured and this serves as reference in the calculation of the amount of the tumor when the percentage of cancer area is evaluated.

DNA ploidy

DNA ploidy analysis of prostate cancer by flow cytometry and digital image analysis provides important prognostic information that supplements histopathologic examination. Controversy exists, however, about whether it is a significant predictor above Gleason score in multivariate analysis. DNA ploidy pattern correlates with cancer grade, tumor volume, and stage. Most low-stage tumors are diploid, and high-stage tumors are nondiploid, but many exceptions occur.

Patients with diploid tumors have a more favorable outcome than those with aneuploid tumors; for example, among patients with lymph node metastases treated with radical prostatectomy and androgen-deprivation therapy, those with diploid tumors may survive 20 years or more, whereas those with aneuploid tumors die within 5 years. The 5-year cancer-specific survival is about 95% for those with diploid tumors, 70% for those with tetraploid tumors, and 25% for

those with tumors that have multiple aneuploid cell lines. However, the ploidy pattern of prostate cancer is often heterogeneous, creating potential problems with sampling error.

Until recently, flow cytometry was the most common method of DNA ploidy analysis for prostate cancer, but it was limited by the need for a large amount of tissue with a great number of cells. The minimum amount of needle-core tissue necessary to yield satisfactory results with flow cytometry is a 0.2-cm length of malignant acini, which corresponds to about 2,500 to 5,000 nuclei.

Digital image analysis overcomes this limitation and is gaining popularity despite a lack of standards for this method. Concordance of digital image analysis on needle biopsy and flow cytometry from radical prostatectomy specimens is 82%. Sensitivity is 87% and specificity is 74% in predicting the presence of cancer. An international DNA Cytometry Consensus Conference reviewed the literature in 1993 and concluded that the clinical significance and biologic basis of DNA ploidy needed further investigation.

Perineural invasion before radical prostatectomy

Perineural invasion, which occurs in up to 38% of biopsies, is common in adenocarcinoma and may be the only evidence of malignancy in a needle core. This finding is strong presumptive evidence of cancer but is not pathognomonic because it occurs rarely with benign acini. Complete circumferential growth, intraneural invasion, and ganglionic invasion are present only with cancer, with rare exceptions. Perineural invasion usually indicates tumor spread along the path of least resistance; it does not represent lymphatic invasion.

Only half of patients with perineural invasion on biopsy have extraprostatic extension. In univariate analysis, perineural invasion was predictive of extraprostatic extension, seminal vesicle invasion, and pathologic stage in patients treated by radical prostatectomy. In multivariate analysis, however, perineural invasion had no predictive value after consideration of Gleason grade, serum PSA level, and amount of cancer on biopsy.

Perineural invasion has limited utility as a diagnostic test for the prediction of extraprostatic extension, with a sensitivity of only 51%, a specificity of 71%, and a positive predictive value of 49%. The negative predictive value was 71%, showing that the absence of perineural invasion was associated with extraprostatic extension in 29% of cases; perineural invasion on biopsy is even less useful as a diagnostic test for the prediction of seminal vesicle invasion.

Perineural invasion is a significant independent predictive factor for adverse outcome at 3 years for patients treated by external beam radiation therapy; however, its value was associated only with a pretreatment PSA level of less than 20 ng/ml, suggesting that the poor prognosis associated with a high PSA level overrides any additional information that perineural invasion may provide.

Microvessel density

A significant increase in microvessel density (MVD) occurs in prostatic intraepithelial neoplasia and carcinoma compared with normal prostatic tissue. Mean blood vessel count is higher in tumors with metastases than in those without metastases, and most studies, but not all, show a correlation with pathologic stage. MVD appears to be an independent predictor of cancer progression in some studies. Increased MVD in prostatic carcinoma is probably related to the production of angiogenesis-associated growths, such as vascular endothelial growth factor or VEGF, which is similar to what occurs in other organs. The cumulative data suggest that increased MVD contributes to extraprostatic spread of adenocarcinoma, perhaps by facilitating microvascular invasion.

Vascular/lymphatic invasion

Microvascular invasion consists of tumor cells within endothelial-lined spaces. The presence of a cellular reaction in the adjacent stroma is not required for diagnosis. Also, we do not differentiate between vascular and lymphatic channels because of the difficulty and lack of reproducibility among different observers by routine light microscopic examination. Microvascular invasion may be confused with perineural invasion and fixation-associated retraction artifact of acini.

Microvascular invasion is present in 38% of radical prostatectomy specimens with cancer. It is commonly associated with extraprostatic extension and lymph node metastases (62% and 67% of cases, respectively), and its presence correlates with histologic grade. Also, microvascular invasion appears to be an important predictor of outcome: it carries a fourfold greater risk of tumor progression and death. It is not an independent predictor of progression, however, when stage and grade are included in the analysis.

The Cancer Committee of the College of American Pathologists recommends reporting microvascular invasion in all prostatic specimens, presumably using routine light microscopic examination, but this recommendation is rarely followed in practice. Immunohistochemical stains directed against endothelial cells such as factor VIII-related antigen, Ulex europaeus, CD31, or CD34 may increase the detection rate.

Other predictive factors

Other preoperative factors may improve the predictive accuracy for pathologic stage, including androgen receptors, nuclear and nucleolar morphometry, neuroendocrine (NE) differentiation, and polymerase chain reaction-based assay of PSA-synthesizing cells in the peripheral blood. These factors basically correspond to those of the CAP category 3.

Prostate cancer may show divergent differentiation towards a NE phenotype in the form of NE small cell carcinoma or carcinoid-like tumors. Much more common is focal NE differentiation in prostate cancer which may be pronounced in approximately 10% of carcinomas. The prognostic significance of focal NE differentiation is controversial but current evidence suggests an influence on prognosis related to hormone resistant tumors and/or a role in the conversion to a hormone resistant phenotype. Chromogranin A appears to be the best overall tissue and serum marker of NE differentiation.

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HANDLING AND REPORTING OF THE RADICAL PROSTATECTOMY SPECIMENS
WORKSHOP IV PART B

E. Eleftheriadis
Histocytopathology Laboratory, Institution for Social Insurance, Prefecture Unit of Health Thessaloniki, Greece.

The prognosis of patients suffering from prostatic adenocarcinoma is correlated to the volume of localized disease, the local and distant extent of the disease and the grade of the tumor

Various methodologies, concerning partial or total sampling and more than one reporting protocols of the radical prostatectomy specimen have been described

All of these guidelines focus mainly on the information that is important to be included in the histopathology report, leaving the choice of specimen handling to the single histopathologist. Nevertheless a noteworthy point in practically all of the studies is that the thoroughness of sampling may affect the assessment of the pathologic staging

Gross description:

- Indication of the anatomic structures that comprise the specimen (i.e. prostate, seminal vesicles and vasa deferentia)
- Separate measurement of the anatomic structures, especially the prostate gland, in three dimensions
- Weighing of the specimen (all of the above should be performed preferably prior to fixation)
- Coating of the entire external surface of the specimen with India ink.
  Avoid inking the distal urethral orifice, the smooth surface of the bladder neck, including the proximal urethral orifice and finally into any surgical incisions
  Different color dyes may be utilized optionally to coat the left and right lobes.

Dissection:

- Remove the most apical section of the prostate, including the distal urethral orifice (approximately 3-4mm) cutting perpendicular to the prostatic urethra.
  Lay the section flat and divide into right and left halves. Serially section each half so that perpendicular sections of the distal apical margin are obtained.
  Submit these sections as RAM (Right Apical Margin) and LAM (Left Apical Margin).
- Shave the soft tissue of the bladder neck at the junction of the rough prostatic capsule and the smooth bladder neck thus obtaining multiple small tissue fragments. Submit these fragments as BNM (Bladder Neck Margin).
- Amputate the seminal vesicles and vasa deferentia from the prostate gland.
  From this amputated section, shave the distal vasa deferentia margins.
  Take one section of seminal vesicle from the end that was amputated from the prostate. Submit these sections as LSV (Left Seminal Vesicle and Vas Deferens) and RSV.
- Serially section the prostate from apex to base,perpendicular to the prostatic urethra, at approximately 3 mm intervals.
  At this point evaluate and make note of the location, size, color and texture of any suspicious lesions or nodules.
  Using the prostatic urethra as a guide divide into right and left halves.
  Using the verumontanum as a guide, separate the slices of each half into anterior and posterior quadrants.
  If a section of the prostate is too large to fit into a single cassette divide into anterior and posterior portions
- Entirely submit the prostate labeling the sections A, B, C from apex to base.
  For example, the apical section of the right and left halves should be submitted as RA (Right prostate, section A) and LA (Left prostate, section A), respectively.
  For sections divided into anterior and posterior portions, the designations A and P should be used. For example, a section taken from the mid-portion of the right half of the gland and divided into anterior and posterior sections should be submitted as RDA (Right prostate, Section D, Anterior section) and LDA.
  Make note of the range of sections consisting the anterior and posterior quadrants of each lobe respectively.
- The seminal vesicles need not be entirely submitted.

Reporting Radical Prostatectomies:

The information suggested to be included in the pathology report, mainly based on present literature, has as follows:

- Gleason Grade (reported always as Gleason score i.e. 3+4=7/10).
- Location of tumor(s) and location of dominant tumor mass (index tumor).
- Pathologic Stage
- Extent of extraprostatic extension, if present
- Margin status, including bladder neck and prostate apex
- Present and extent of high grade PIN
- Status of seminal vesicles
- Lymph node status
- Treatment related changes

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