Parallel Symposia

SY-1. Genetic Aspects and Molecular Pathology (September 11)

SY-1-1 Quality Control in Molecular Pathology

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Somatic alterations to key regulatory genes — oncogenes & anti-oncogenes — act additively in the genesis of tumors and can be measured in tumor biopsies. Research on the molecular genetics of cancer has invaluable cognitive importance and shows some major impacts for cancer treatment: first, some oncogenes or anti-oncogenes endow cancer cells with a special phenotype, hence alteration to certain oncogenes display prognostic and predictive value, as in the case of *erb*B2 in breast cancer. Second, inheritance of mutated alleles of certain genes predispose to the development of specific types of cancer, so that detection of those alleles may select out people to special preventive strategies. Lastly, the characterization of the leading molecular change in a tumor is the necessary step to devise a specific gene therapy. Molecular biology has introduced spectacular methodologies, which allow evaluation of gene deletion, amplification, rearrangement, single-base mutations and expression, even when few tumor cells are available. However, it is common to observe in the literature large discrepancy between different studies, especially when considering statistical aspects, e.g. the frequency of a specific mutation in a given type of cancer or the frequency of cases overexpressing a given oncogene. This is mainly due to differences in the methods and reagents employed, which are usually drawn directly from the basic research lab and display large individual-based variation. More and more frequently, molecular pathology is being involved in large-scale clinical applications. In breast cancer, this is well illustrated by the cases of *erb*B2, *p53, myc*, BRCA 1 & 2. A review on the myriad of reports on *erb*B2, for example, reveals impressive variation in frequencies and correlations, as measured by distinct methodological approaches. Clinical use of molecular pathology requires that multicentric comparative methodological trials be run and that adequate qualitative & quantitative reference standards be implemented.

SY-1-2 Genes Predisposing to Breast Cancer

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Much progress has been made over the past few years in identifying breast cancer predisposing genes. Three genes have been identified that confer high risks, i.e. *TP53, BRCA1,* and *BRCA2.* Germline mutations in *TP53* are associated with the Li-Fraumeni syndrome, and are presumably very rare. Female mutation carriers of *BRCA1* have been estimated to have an 87% risk to develop breast cancer before the age of 70, and 63% risk to develop ovarian cancer before that age. Similar breast cancer risks are predicted for *BRCA2,* while this gene is also particularly associated with breast cancer in males. The existence of at least two moderate risk genes is now also clear. Recessive heterozygous mutations in the gene for Ataxia telangiectasia *(ATM)* are expected to be present in 2–7% of all breast cancer cases, but have been estimated to confer only moderately increased risks for the disease. *CDS,* for Cowden's Syndrome, is rare in the population and its associated lifetime risk of breast cancer is approximately 30%. *BRCA1* and *BRCA2* are thus peculiar in that they are predicted to be relatively frequent in the general population and to confer high breast cancer risks. Finally, it is becoming increasingly evident that other genetic factors may modify the expressivity or penetrance of these genetic factors. One of them appears to be the variable number of tandem repeat (VNTR) locus of *HRAS1. BRCA1* carriers with a history of ovarian cancer were found to carry one or two rare alleles at this locus more frequently than carriers without ovarian cancer (odds ratio 2.85: P = 0.002).

SY-1-3 Loss of Heterozygosity and Prognosis

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The etiology of breast cancer involves a complex interplay of various factors, including genetic alterations. Many studies have been devoted to the identification and characterization of mutations that occur frequently during breast tumorigenesis. The major types of genetic abnormalities frequently observed in breast tumor DNAs are amplification of proto-oncogenes (*MYC, ERBB2*) and chromosome band 11q13, mutation of *TP53,* and loss of heterozygosity (chromosomes and chromosome arms 1, 3p, 6q, 7q, 8p, 11, 13q, 16q, 17, 18q and 22q). The latter may correspond to losses or inactivations of tumor suppressor genes.

Certain of these genetic anomalies appear to be of prognostic value in clinical oncology. The principal alterations of potential prognostic value are amplification and overexpression of the *MYC* and *ERBB2* proto-oncogenes and of the amplification unit 11q13, as well as alterations in the *NME1* and *TP53* suppressor genes (see review by Gasparini et al., 1993). However, this first list of alterations is provisional, since the identification of deletions associated with a poor prognosis, such as those in 7q31, 16q and 17p13.3, suggests that other tumor-suppressor genes may be of prognostic value.

Identification of these putative suppressor genes and their confirmation as novel factors of great prognostic value will require further studies.

SY-1-4 P53 Mutations and Other Genetic Changes in Invasive and Intraductal Breast Cancer

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Knowing the genetic alterations present in breast cancer (and the alterations in protein expression resulting from these alterations) may help in predicting clinical behaviour and guiding therapy. Using immunohistochemistry, we have investigated paraffin embedded tumour specimens from 441 node-negative premenopausal patients who were randomized in a trial comparing perioperative chemotherapy with no adjuvant therapy for the expression of p53, c-erbB-2/neu, estrogen- and progesterone receptor and Ki-67. Patients with p53 negative staining tumours showed a benefit from adjuvant chemotherapy ($P < 0.01$), whereas patients with p53 positive staining tumours did not.

Ductal carcinoma in situ (DCIS) is heterogenous with respect to histologic type, clinical presentation and clinical behavior. In 120 cases of DCIS, p53 was overexpressed in 26% of cases, c-erbB-2/neu overexpression (as a result of gene amplification) in 46% of cases and for both was associated with the poorly differentiated type of DCIS, which is more likely to progress to invasive carcinoma. Loss of heterozygosity for loci on chromosome 16 was associated with well differentiated DCIS, whereas LOH on chromosome 17 was associated with poorly differentiated DCIS.

SY-2. Ductal Carcinoma In Situ (DCIS) (September 11)

SY-2-1 The Classification of Ductal Carcinoma in Situ of the Breast

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Ductal carcinoma in situ (DCIS) is a heterogeneous lesion being diagnosed with increasing frequency as it is often detected by mammography. Treatment by conservation therapy is still controversial. Success may depend on several factors, including lesion size, adequacy of excision and histological type. Traditional histological classification is unsatisfactory. There is considerable interest in establishing alternative criteria based on nuclear features,

cell polarization or necrosis and several new classifications have recently been proposed. Careful correlation of these classifications with other features of DCIS and, most importantly, development of invasive carcinoma and death from metastatic carcinoma following conservation therapy is of the utmost importance for development of future strategies for management of this heterogeneous lesion.

SY-2-2 Imaging of DCIS for Diagnosis and Treatment

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Before the widespread use of screening mammography DCIS was considered a relatively uncommon lesion, accounting for 0.8-0.5% of all breast carcinomas. In recent studies of patients undergoing mammographic studies without any clinical abnomaly, DCIS accounts up to 15–20% of all breast cancers. Although clustered microcalcifications are a highly sensitive mammographic sign of DCIS, its specificity ranges from 10 to 35%. As a consequence, the number of diagnostic surgical biopsies for clustered microcalcifications has dramatically increased. Furthermore, the extent of DCIS cannot always be accurately predicted on mammograms. Therefore, the diagnosis and assessment of DCIS on mammograms are one of the most common and difficult challenges for the radiologist. The diagnostic accuracy of core needle biopsy for isolated clustered microcalcifications associated with DCIS is an issue that is still debated. More recently, several series reported contrast enhancement in DCIS up to 95% on dynamic MRI. Furthermore, the extent of contrast enhancement statistically correlated with the histopathologic analysis. If further studies confirm these results, dynamic MR could prove to be an useful tool for diagnosing and planning local treatment of DCIS.

SY-2-3 SY04 Ductal Carcinomas in Situ: Clinical Trials

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The treatment of DCIS is a source of perplexity and more and more cases are diagnosed as a result of the development of mammography and screening programs. A simple mastectomy will cure almost 100% of cases, the few failures being due to a small invasive carcinomas missed by the pathologist. However it is difficult to justify such a mutilating operation for DCIS that are often now very small lesions, while, invasive carcinomas are treated conservatively. But which conservative treatment and for which DCIS? First, what is the role of radiotherapy after a complete wide excision? Several national trials have started in Europe and also the EORTC 10853 and in the USA the NSABP B17 which early results have been published. In the EORTC trial Lesions up to 5 cm will have *a complete excision* and are randomized to: Radiotherapy to the whole breast 50 gy (2 gy × 25 in 5 weeks) or not. Strict guidelines are given for the quality of the excision, a quality control on site and a central review of histopathology are done. Ineligible cases are also registered. Thus we collect a large data base that will provide precious resources of clinical, pathological and biological information which enable markers of risk of relapse to be identified. This may lead to a more individualized approach to management of DCIS with some patients being treated with local surgery alone, other needing additional radiotherapy and some needing mastectomy. Soon it should be possible to treat DCIS on a basis of precise information for clinical trials rather than a result of clinical prejudice.

SY-2-4 DCIS: Quality Control of Conservative Treatment

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DCIS is diagnosed after two different clinical situations. Firstly, DCIS is suspected by microcalcifications on routinely performed mammography. Secondly, DCIS is — coincidently — found in a breast biopsy performed for any symptomatic reason, usually being a breast mass or bloody nipple discharge. The situation of clinical occult microcalcifications poses the clinician for more difficult questions: how to diagnose the nature of the calcifications, do they represent only in situ carcinoma or also invasive cancer, what is the extent of the disease and consequently how to proceed to — and not jeopardizing — optimal treatment? For the diagnostic work-up a high quality two-directional mammography with magnification views should always be available. Concerning the microscopical diagnosis, stereotactic FNA-cytology is insufficiently reliable to establish the diagnosis of DCIS. Multiple stereotactic core biopsies enables the exclusion of a malignancy if there is a complete concordance between the mammographical appearance and histological findings. Core biopsy does not give information about extent and infiltration. Furthermore, uncertainties exist about the risk of needle tract seeding, and spill in case of hemorrhage.

In the absence of core biopsy diagnosis, diagnosis has to be made after wire guided biopsy. The wire localization has to be very precise. This biopsy, aimed at diagnosis, has to be representative, and should not exceed a lump of 30–40 grams (4x4x4 cm). Per operative specimen radiography is mandatory, as is identification of the specimen for the pathologist. Frozen section histology is strongly discouraged. The pathologist should examine the specimen fully, inking of the margins is indispensable and the performance of multiple slides (2–3 mm) radiography is very helpful. For reliable exclusion of invasion and margin involvement, on average 10 slides have to be examined. If margins are close, or if there are any doubts on the completeness of the excision of the mammographical microcalcifications, a post-operative mammogram should be made, preferably 6–8 weeks later (there is no need for haste in DCIS!). As the treatment of DCIS is aimed at complete local excision, in case of presumed incomplete excision a re-excision, if indicated with a guide wire re-localization, has to be performed. Quality in the management of DCIS is only controlled within a dedicated "breast team": a radiologist with high quality mammography and localization equipment, a surgeon with knowledge of the biological nature of DCIS, a pathologist who is prepared to a complete work-up. In any case, local control should be comparable to that after total mastectomy: less then 10% chance of breast relapses (half of them invasive!) in 10 years.

SY-3. Local Recurrences After Breast Conserving Therapy (September 11)

SY-3-1 Local Recurrences after Breast Conserving Therapy: Tumor Characteristics

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About 60% of invasive breast cancers are multifocal, of which approximately 15% have a very high multifocal tumor burden, usually of the intraductal type (extensive intraductal component; EIC), outside the invasive mass. On the other hand, about 40% of invasive breast cancers are unifocal, having no tumor foci beyond the index tumor. Optimal therapy may vary for these groups of cancers from breast-conserving surgery with or without local radiotherapy to mastectomy. This therapeutic choice is largely based on the expected amount of residual tumor left behind after an excisional biopsy.

Optimum pathology supported by specimen X-ray and high quality mammography is capable to predict the type and amount of potential residual tumor in most of the cases.

In a recent study on a series of 149 patients, the amount of DCIS within 1 cm from the invasive edge of the primary, and the size of margin-width around the primary turned out to be the most important factors in the assessment of residual tumor after an excisional biopsy. Therefore, at histologic evaluation the attention should be focused primarily on the assessment of the amount of DCIS around the invasive tumor and the relationship of these tumor foci to the margins.

An adequate pathologic evaluation of the margins requires a proper orientation of the specimen during handling by the pathologist.

SY-3-2 Local Recurrences after Breast Conserving Therapy: Therapeutical Factors

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Many risk factors for local recurrence after breast conserving treatment are connected with surgical procedure, irradiation dose and technique, adjuvant treatment. The purpose of our study is to report on the basis of the literature and of our experience, the role of some therapeutical factors in the occurrence of local recurrences. In the period 1980–1992 1574 patients with breast cancer (T < 3 cm) were treated with conservative treatment (surgery + radiotherapy) in our Hospital. Mean and median follow-up is 79 and 84 months respectively. The actuarial incidence of breast recurrences at 8 years was 7.1 ± 1%. pT1 (1185 pts) vs. pT2 (374 pts): 6.8 ± 1.1% vs. 7.8 ± 2% NS. Negative margins (898 pts) 6.1 ± 1.6% positive margins