

LECTURE 5

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Molecular and cytogenetic analysis of solid tumors

In this lecture two-part lecture we will focus on the importance of cytogenetics and molecular biology in understanding the causation of solid tumors and on how this information is being developed for the clinical laboratory. We will review the recurrent rearrangements in some of the pediatric neoplasms and relate the observed changes to the molecular biology that underlies these cancers. The role of cytogenetics, FISH and molecular diagnostics as performed in Toronto will be reviewed using the management of Ewing and rhabdomyosarcomas as examples. The research applications of comparative genomic hybridization (CGH) and spectral karyotyping (SKY) will be discussed drawing on examples from brain tumors, osteosarcoma, retinoblastoma, ovarian and prostate cancer.

Slide 1 TITLE Importance of fusion oncoproteins in the development of sarcomas

TEXT AS A TABLE

- Sarcomas are soft tissue (mesoderm derived) tumors affecting a number of different sites.
- Each one seems to have a consistent translocation observed in the majority of tumors.
- We will look at Ewing sarcoma, rhabdomyosarcoma and osteosarcoma.
- These tumors show increasing cytogenetic complexity and illustrate how cytogenetics and FISH methods can be very helpful.

Slide 2 TITLE: Short term culture of Ewing sarcoma

IMAGE OF CULTURE OF A EWINGS SARCOMA

- Bone derived tumor very similar if not identical to pPNET (site difference)
- Both express MIC2 and neural markers
- Both have the 11;22 reciprocal translocation involving EWS and FLI-1 genes the

Slide 3 TITLE: Ewing sarcoma karyotype

IMAGE OF KARYOTYPE

- Primary change is 11;22 translocation
- Secondary acquired changes in this tumor are trisomies of 2 and 8
- 50% of tumors have trisomy 8.
- Variant translocations also seen

Slide 4 TITLE: Ewing sarcoma and pPNET

TEXT

- t(11;22)(q24;q12)
- 90% of Ewing and pPNET have this aberration
- chimeric protein 5' EWS on 3' end of FLI-1
- EWS is RNA binding domain
- FLI-1 DNA binding
- t(21;22) seen in 8% cases
- fuses ERG on 21 to EWS on 22

Slide 5 TITLE: Rhabdomyosarcoma (RMS)

IMAGE OF HISTOLOGY AND KEY POINTS ABOUT THESE TUMOR SUBTYPES

- malignancy of skeletal muscle. Both express muscle markers such as MYOD1

- alveolar has more open histological nests of cells than tighter packed embryonal RMS
- alveolar RMS more aggressive tumor therefore important to distinguish them
- alveolar RMS has 2;13 translocation embryonal does not
- embryonal RMS has LOH at 11p15 –may involve IGF2 gene. Not usually seen in alveolar RMS
- alveolar RMS has N-myc amplification (about 50% of this subtype) embryonal RMS does not

Slide 6 TITLE: Alveolar RMS

IMAGE OF KARYOTYPE

- Long chromosome is the t(2;13) translocation
- This tumor must have had the translocation in a diploid progenitor cell which subsequently underwent tetraploidization (whole chromosome set doubling due to a failure of cytokinesis) since it has duplicate copies of the primary aberration.

Slide 7 TITLE: Alveolar RMS

TEXT

- Translocation seen in 70% of cases. PAX 3 on 2 and ALV on 13
- both fusion proteins have DNA binding properties-majority of fusion oncoproteins are dysregulated DNA binding proteins indicating that disruption of gene control is major path in cancers.
- Variant involves PAX 7 gene instead of PAX3

Slide 8 TITLE: Embryonal RMS

TEXT

- No consistent change
- LOH seen at 11p15
-

Slide 9 TITLE: LOH of IGF2 in RMS

IMAGE OF PCR gel

- PCR of patients blood shows heterozygous 1,2 pattern
- Tumor shows only 2,2 pattern indicating deletion or more likely mitotic recombination (as discussed for RB earlier)

Slide 10 TITLE: Osteosarcoma (OS)

TEXT

- Peak incidence between 10-19 (distinct adult form in later life)
- 2.1 case per million per year (v. rare)
- 80-90% occurs in long bone
- serum alkaline phosphatase levels- tumor activity
- aneuploid karyotypes
- molecular changes p53, MDM2 RB1

Slide 11 TITLE: OS

IMAGE OF TUMOR IN LONG BONE (X-RAY)

Slide 12 TITLE: Histologic variants in OS

IMAGE OF MAIN HISTOLOGIES

Chondroblastic, Osteoblastic etc. based on differentiation morphology

Slide 13 TITLE: Complex karyotypes seen in OS cell line

IMAGE OF OS KARYOTYPE

- many aberrations will be pointed out.
- Some will have been induced by in vitro growth others were present in vivo.

Slide 14 TITLE: MORE COMPLEX

IMAGE OF OS KARYOTYPE

- Many markers. Marker = chromosome in which some or all of the region is so rearranged by structural changes that the banding pattern cannot allow identification of the chromosomal origins

Slide 15 TITLE: Spectral karyotyping

IMAGE OF SPECTRAL KARYOTYPING (SKY) MICROSCOPE

- Recently, universal chromosome painting techniques have been developed in which it is possible to analyze all chromosomes simultaneously. SKY is based on the principle of the differential display of colored fluorescent chromosome-specific paints providing a complete analysis of the human chromosomal complement. Using combinations of 23 different colored paints as a "cocktail probe", subtle differences in fluorochrome labelling profiles after hybridization with this cocktail allows the computer to assign a unique colour to each chromosome pair
- The large camera shown in this slide can register the full spectrum of paint signals pixel by pixel and are generated as shown schematically on the next slide.

Slide 16 TITLE: Schematic overview of SKY method

SCHEMATIC

In this example a pure blue paint is derived only from chromosome 1 which is then false colored in yellow by computer. The middle part of this abnormal chromosome is a 50% mixture of both blue and red dyes but the computer assigns a red color to these regions and finally the pure red dye is classified as green.

Thus, abnormal chromosomes in the karyotype of a tumour can be identified by the pattern of colour distribution along the axis of the chromosome so that rearrangements between different chromosomes will lead to a distinct transition from one colour to another at the position of the breakpoint. SKY technology is particularly suited to solid tumours where the complexity of the karyotypes (such as in OS) may often mask the presence of subtle chromosomal aberrations.

Slide 17 TITLE: OS SKY karyotype

IMAGE

Slide 18 TITLE: SKY method

TEXT and SCHEMATIC DIAGRAMS

- make slide in usual way
- purchase cocktail probe (already labelled)
- denature slide and probe then suppress repeats using COT1 repetitive DNA blocker
- Hybridize for two days then wash and acquire and analyze using SKY computer

Slide 19 TITLE: SKY analysis of Ewings

IMAGE

Extra aberrations in addition to 11;22 can be seen (on the right)

May be clinical implications of more complex karyotypes

Slide 20 TITLE: - Outcome analysis of Ewings

GRAPH OF PATIENT SURVIVAL

More complex karyotypes of the type just seen indicate a less favorable response to treatment.

Slide 21 TITLE: Chromosome Analysis of another OS by SKY

IMAGE

SKY karyotype shows a haploid number of chromosome

Slide 22 TITLE: Chromosome Analysis of OS by SKY draws attention to chromosomes 4 and 6
More OS tumors needed to continue this study. Please help if you know of OS tumors that could be made available for our study!

DNA available from more OS tumors so comparative genomic hybridization (CGH) is possible

Slide 23 TITLE: CGH

TEXT

“A method capable of detecting and mapping relative DNA sequence copy number between genomes... a copy number karyotype can be generated for a tumor by the comparison of DNAs from the malignant and normal cells thereby identifying regions of gain or loss of DNA”

Slide 24 TITLE: - CGH Methodology

SCHEMATIC

- In typical CGH experiments DNA from malignant and normal cells such as fibroblasts is labeled with two different fluorochromes and then hybridized simultaneously to *normal* chromosome metaphase spreads. Tumour DNA is labeled with biotin and detected with fluorescein (green fluorescence); the control DNA is labelled with digoxigenin and detected with rhodamine (red fluorescence). Regions of gain or loss of DNA sequences in the tumour, such as deletions, duplications, or amplifications, are seen as changes in the ratio of the intensities of the two fluorochromes along the target chromosomes. In this example chromosome 1 has gains (all green), chromosome 2 has gene amplification near the top (small regions of intense green) and chromosome 6q has a deletion (red)

Slide 25 TITLE: - Chromosome Analysis of GG

SCHEMATIC SHOWING EACH CHANNEL: RED =NORMAL, GREEN = TUMOR, BLUE = DAPI (inverted DAPI IS LIKE G-BANDING)

An amplified sequence will generate increased green fluorescence, whereas a deletion will shift the red/green ratio towards red. For low copy number amplifications and hemizygous deletions, this change in fluorescence ratio is difficult to distinguish by eye and requires specialized image analysis software. Only tumor DNA needed to do CGH. Chromosomes used derive from normal donors. One disadvantage of CGH is that it can only detect large blocks (>5 Megabases) of over- or under-represented chromosomal DNA; and balanced rearrangements such as inversions or translocations escape detection.. Since 1992 CGH has gained wide acceptance as a new and promising approach for understanding the complex cytogenetic changes in solid tumours.

Slide 26 TITLE: - CGH Profiles from a neuroblastoma

IMAGE OF PROFILE FROM ANALYSIS OF 9-10 CELLS INTEGRATED

Notice where red: green ratio goes away from mid point. Thus chromosome 1 is gained , 2p amplified, 3 is normal, 6q is deleted, 17 q is gained etc.

Slide 27 TITLE: - CGH analysis of a medulloblastoma
IMAGE OF PROFILE FROM 5-6 CELLS INTEGRATED

This is a much more complex CGH profile indicating the cytogenetics from this case would have been very abnormal if we had been able to study them by conventional methods

1p is normal, 1q is gained, 2p24 is highly amplified, 3q is gained, 4p is gained and 4q is lost (this suggest an isochromosome of 4p may have formed etc..

Please ask more questions in the break as it is critical that the principles behind this technique are understood.

----- COFFEE BREAK-----

Slide 28 TITLE: - Isochromosome 6p analysis seen in retinoblastoma (RB)

-This change in which a centromere misdivision leads to gain of 6p is very common in RB
-why?

Slide 29 TITLE: - CGH Frequency of mutations in RB tumors

- 6p is very common in RB and quite specific
- the other changes are interesting but the interest of our group has been on understanding the specificity of gain of 6p
- Sometimes a partial gain is seen-important clue!

Slide 30 TITLE: - Chromosome 6p paint study of RB tumors

- Dr Gallie only has DNA on most tumors so painting or SKY not possible
- working with Danian Chen (a visiting fellow from China) and Brenda Gallie we looked at 47 tumors by CGH

Slide 31 TITLE: - CGH study of 47 RB tumors

- Losses are grouped on left as vertical bars.
- Gains on right
- Yellow shows clusters of gain to follow up on
- Blue shows recurrent losses
- As expected not much change on chromosome 13
- 1q seen in many different classes of tumor (not RB specific)
- 2p probably N-myc
- gain of 6p and loss of 16q very interesting

Slide 32 TITLE: - minimal regions of gain in 6p and loss in 16q

- more recently Dr Chen has identified one gene in this region using genomics methods
- gene will likely be an oncogene
- genes on right could be tumor suppressor genes for involvement in progression in RB after primary changes of RB gene have taken place.

Slide 33 TITLE: - Ovarian cancer schematic

Analysis of acquired changes in tumors

New project – little know and about the chromosomes in this tumor

We will review microarray methods in tomorrow's lecture

Slide 34 TITLE: - Ovarian cancer

Leading cause of death from gynecological malignancies.

Fourth

Leading cause of death from gynecological malignancies.

Fourth leading cause of cancer death among North American women.

Response to treatment is poor.

Molecular mechanisms for both INHERITED and SPORADIC cancers are largely unknown.

What we know so far:

Loss of BRCA1 (17q21) or BRCA2 (13q12) - Inherited

p53 inactivation by allelic loss (Serous tumours?)

PTEN inactivation (Endometrioid tumours?)

Over expression of oncogenes including: fms, HER2/NEU

Classical cytogenetics have revealed complex karyotypes and non-random aberrations of chromosomes 1, 3, 6, 11, 12 and 19.

Comparative Genomic Hybridization has identified new regions of gain and loss specific to ovarian cancer.

Slide 35 TITLE: - Ovarian cancer SKY from Ascites Fluid

Slide 36 TITLE: - Ovarian cancer SKY

Slide 37 TITLE: - Ovarian cancer SKY and CGG analysis of same tumor

Notice gain of 1q and change in 3 using both techniques/ CGH allows us to work out whereabouts additional SKY signal comes from

Slide 38 TITLE: - Summary of CGG analysis of all 11 ovarian tumors

Red is loss and green is gain.

Slide 39 TITLE: - Ovarian cancer SKY and CGG analysis results combined

SKY analysis failed to detect any recurrent structural aberrations. Aberrations were present at a rate of 19.1 aberrations per tumor (range 8 – 29) for the untreated group of tumors in the regions 3p13-p21, 3q11-q12, 3q22-q23, and 8q23-q24 and parts of 6, 11 and 17 had elevated frequencies of structural and numerical aberrations by SKY and CGH. Each of the histological subtypes is shown by the colors indicated. The black dots are SKY breakpoints.

Tomorrow we will review microarray studies of this same tumor group.

Slide 40 TITLE: - Introduction Prostate cancer (CaP) cytogenetics

Prostate cancer (CaP) has the leading cancer incidence in men in N. America; 2nd most common cause of male cancer mortality.

While localized CaP is treatable, advanced androgen-independent disease is incurable and terminal. Critical need for a molecular progression marker(s). is treatable, advanced androgen-independent disease is incurable and terminal. Critical need for a molecular progression marker(s) such as chromosomal changes.

Slide 41 TITLE: - Prostate cancer (CaP) cytogenetics

Little is known of prostate cancer cytogenetics the literature is rather confusing. Of the several reported chromosomal changes associated with CaP, including those on chromosomes 7, 8, 10, 13q, 16, 17, 18q, and Y aberrations on chromosome 8 have received the most attention. Allelotyping experiments have demonstrated frequent involvement of chromosome 8 in CaP tumorigenesis, and analysis of extensive loss of heterozygosity (LOH) loci along 8p in CaP Prostate cancer is a multifocal heterogeneous disease. At early stage, the disease outcome cannot be effectively prognosticated based on the histopathology, and at present, a major challenge in prostate cancer research is to identify early biomarkers which herald aggressive transformation.

Slide 42 TITLE: - Schematic Model of Genetic Events in CaP

prostate cancer genetics suggests a model in which there is slow accumulation of multiple genetic events over time. Clinically, localized CaP is often slow-growing and latent and its diagnosis sometimes may not even impact survival for 10 to 15 years, further complicating disease assessment and prognosis. In a small but significant number of cases, however, the disease progresses to advanced stages. Advanced androgen-refractory disease is ultimately incurable and terminal. Identification of an early stage CaP-specific progression marker will allow delineation of tumor subsets that will stay indolent requiring no clinical intervention, and those that will progress to metastasis. Today we will look at SKY analysis of CaP and tomorrow review how genomic methods are being used to understand progression in CaP.

Slide 43 TITLE: - SKY analysis of PC3

Slide 44 TITLE: - SKY analysis of PC3

Slide 45 TITLE: - SKY analysis of LnCAP

This tumor is androgen responsive and is thought to represent an earlier stage than DU145 and PC3. Direct SKY analysis of fresh CaP from patients has been disappointing. Always normal karyotypes found.

Slide 46 TITLE: - SKY analysis of 1532T – HPV-Immortalized
Less changes and draw attention to chromosome 8

Slide 47 TITLE: - SKY analysis of chromosome 8
Notice how aberrations lead to gain of 8q and loss of 8p
CGH analysis indicated

Slide 48 TITLE: - CGH analysis of CaP
CGH indicates chromosome 8 important

Slide 49 TITLE: - Classical cytogenetics and CGH findings
SUMMARY TABLE

Cytogenetics and CGH studies of CaP that have been published generally have examined late stage, metastatic disease. These studies have shown that there are frequent chromosomal aberrations in late stage CaP tumors, and that chromosomal copy number losses are five times more prevalent than gains.

The histological heterogeneity of CaP means that additional genomic methods are required to understand this complex solid tumor. These methods will be reviewed tomorrow.

Slide 50 TITLE: - Conclusions

SKY can detect subtle changes that could be missed by standard techniques
CGH complements SKY and draws attention to specific regions of loss and gain
Consistent changes found by these methods will be entry points for future molecular genomic approaches to finding specific changes associated with tumors.