Retroviral oncogenesis
Two major mechanisms identified in animals...

- **Retroviral Transduction**
  - mediated by the “acutely-transforming retroviruses”
    - Abelson Murine Leukemia Virus (A-MuLV)
    - Rous Sarcoma Virus (RSV)
  - these retroviruses carry a transduced oncogene

- **Proviral Integration**
  - mediated by the “slowly-transforming retroviruses”
    - Murine Leukemia Virus (MuLV)
    - Mouse Mammary Tumor Virus (MMTV)
    - Avian Leukosis Virus (ALV)
  - normal retroviral genome (e.g., gag, pol, env)

Human retroviruses

- **1977** - Human T-cell Leukemia Virus (HTLV)
  - causes an acute T cell leukemia
  - endemic in Japan and on some Caribbean islands
  - mechanism of HTLV-mediated transformation?
    - not retroviral transduction
    - not proviral insertion
  - the mechanism is poorly understood. May involve the HTLV regulatory proteins, *tax* and *rex*.

- **2006** - Xenotropic MuLV-related virus (XMRV)
  - implicated in human prostate cancer, but remains controversial.
Oncogene Hypothesis - alterations in a specific subset of cellular genes (the proto-oncogenes) can promote cancer formation.

although the mechanisms of retroviral-induced tumorigenesis are not directly relevant to human cancer, these studies served to identify a large number of proto-oncogenes.

Many proto-oncogenes identified in studies of retroviral tumorigenesis in animals

➔ Are these proto-oncogenes involved in human cancer?
Oncogenes II

- Organization of today’s lecture
  - *in vitro* gene transfer experiments
  - *in vivo* gene transfer experiments
  - chromosome abnormalities
  - chromosome abnormalities in lymphoid tumors
  - chromosome abnormalities in solid tumors
  - gene amplification
  - the proteins encoded by proto-oncogenes
  - targeted cancer therapy (e.g., the Ph¹ chromosome)

Retroviral infection of chick embryo fibroblasts (CEFs)

- almost every RSV virion can transform a fibroblast to yield a visible focus.
  - # of foci is proportional to # of RSV virions
  - provides an ideal method to titer RSV

- high efficiency reflects the activities of:
  - viral surface protein (which facilitates infection)
  - viral integrase (which facilitates proviral integration)

→ efficiency of transformation: ~ 1 focus/cell
  (at saturating quantities of virus: # virions >> # fibroblasts)
Transfection of CEFs with proviral DNA

- donor DNAs: the RSV provirus
  the ALV provirus (control)
  • note: apply saturating quantities of DNA to cultured cells.
    (# of proviral DNA molecules >>> # of fibroblasts)

- Some cells are transformed by the RSV (but not the ALV) proviral DNA to yield visible foci

- This is a less efficient process than viral infection:
  • transfection vs. infection (perhaps 1/10 cells are transfected)
  • proviral integration without integrase (perhaps occurs in only 1/10^2 of the transfected cells)

→ efficiency of transformation: ~ 1 focus/10^3 cells

CEF transfection with chicken genomic DNA

- donor DNA: the genome of uninfected cell
  " " " ALV-infected cell
  " " " RSV-transformed cell

- a few cells are transformed by genomic DNA of an RSV-transformed cell

- this is an inefficient process
  • transfection vs. infection (perhaps 1/10 cells are transfected)
  • proviral integration without integrase (perhaps in 1/10^2 of transfected cells)
  • the likelihood that the integrated DNA will include the RSV provirus (perhaps 1/10^2)

→ efficiency of transformation: ~ 1 focus/10^5 cells
Though seemingly inefficient, the activity of a single oncogene (proviral \( v\)-\( src \)) can be readily detected within the background of an entire cellular genome.

Screening more than \( 10^6 \) cells in a focus formation assay is quite easy.

Can this assay be used to detect and isolate activated proto-oncogenes in human tumors?

Transfection of rodent fibroblasts with human genomic DNA

- Donor DNA - the genome of a human tumor
  - from a tumor cell line or a primary tumor

- Control DNA - genome of normal human cells
  - from blood or other normal tissues
NIH3T3 cells

- a permanent cell line derived from a “post-crisis” culture of normal mouse fibroblasts

- properties of NIH3T3 cells
  - immortal
  - contact-inhibited
  - require anchorage for growth
  - do not form tumors in nude mice

Transfection of NIH3T3 cells with human genomic DNA

- Results of transfection experiment:

<table>
<thead>
<tr>
<th>Genomic DNA</th>
<th>Transformed foci</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal cells</td>
<td>1 / 10^7 cells*</td>
</tr>
<tr>
<td>tumor A</td>
<td>1 / 10^7 cells</td>
</tr>
<tr>
<td>tumor B</td>
<td>1 / 10^5 cells !!</td>
</tr>
<tr>
<td>tumor C</td>
<td>1 / 10^7 cells</td>
</tr>
</tbody>
</table>

- positive results with ~ 20% of human tumors
- seen in a broad spectrum of tumors, including sarcomas, carcinomas, leukemias and lymphomas
New properties of *in vitro*-transformed NIH3T3 cells

<table>
<thead>
<tr>
<th></th>
<th>parental</th>
<th>transformed</th>
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</thead>
<tbody>
<tr>
<td>■ immortality</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>■ focus formation</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>■ colony formation</td>
<td>–</td>
<td>+</td>
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<tr>
<td>■ in soft agar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>■ tumor formation</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>■ in nude mice</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

also, genomic DNA of transformed NIH3T3 cells is active in subsequent *in vitro*-transformation assays (unlike DNA of parental NIH3T3 cells).

Sequential transformation of NIH3T3 cells

- EJ cells human bladder carcinoma
- NIH-3T3 1'-transformant
- 100% human DNA
- ~1% human DNA
- ~99% mouse DNA

- note: the genome of the primary transformants should contain...
  - mostly mouse genomic DNA (~99%)
  - some newly integrated human genomic DNA (~1%)
  - the EJ cell transforming sequences
Sequential transformation of NIH3T3 cells

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<th>~ 1 % human DNA</th>
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<tbody>
<tr>
<td>~ 99 % mouse DNA</td>
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<table>
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<tr>
<th>NIH-3T3 2'- transformant</th>
<th>~ 0.01 % human DNA</th>
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<td>~ 99.99 % mouse DNA</td>
<td></td>
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<th>~ 0.0001 % human DNA</th>
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- the tertiary transformants should contain the EJ cell transforming sequences, but little other human DNA.

What is the transforming agent in human tumor DNA?

- isolate human DNA fragments from tertiary-transformants.
- test each fragment for the ability to transform NIH3T3 cells
  *in vitro*.
- the transforming activity of EJ cells could be ascribed to a single fragment of DNA.
- sequence analysis revealed that the transforming fragment is the c-H-ras gene - a known proto-oncogene that had already been identified in animal studies!!
  - the transduced oncogene of Harvey Sarcoma Virus (HSV), an acutely-transforming mouse retrovirus.
  - the targeted proto-oncogene of retroviral integration in ALV-induced nephroblastomas.
The Ras gene family

- Similar approaches were used to isolate transforming genes from many human tumor cell lines:

  - **EJ cells** bladder carcinoma → **c-H-ras** transduced oncogene of HSV
  - **T24 cells** bladder carcinoma → **c-K-ras** transduced oncogene of Kirsten Sarcoma Virus, another acutely-transforming mouse retrovirus
  - **Lx-1 cells** lung carcinoma → **N-ras** novel member of Ras family
  - **SK-CO-1 cells** colon carcinoma
  - **SK-N-SH cells** neuroblastoma
  - **HL60 cells** leukemia

- Transforming Ras oncogenes were also detected in DNA from primary human tumors

The Ras gene family

- The three members of the Ras family represent most, but not all, of the human oncogenes isolated on the basis of NIH3T3 cell transformation.

- ~15% of all human tumors harbor a Ras gene that has the ability to transform NIH3T3 cells.

- However, Ras genes from normal human DNA do not transform NIH-3T3 cells.

→ the Ras gene of EJ cells is malignantly “activated”
Malignant activation of the Ras genes

- compare nucleotide sequences of transforming Ras genes from human tumors with the non-transforming Ras genes from normal cells.
- Each transforming Ras gene has a point mutation that yields an amino acid substitution at residue 12, 13, or 61.
- Mutation of the same residues is also observed in the retrovirally-transduced Ras oncogenes!!
  - Harvey Sarcoma Virus → v-H-ras (G12R mutation)
  - Kirsten Sarcoma Virus → v-K-ras (G12D mutation)
→ malignant activation of Ras proteins by point mutation !!

Other human oncogenes identified on the basis of NIH3T3 transformation

Many encode protein tyrosine kinases:
- Neu (Her2) - isolated from a neuroblastoma
  - activated by missense mutation
- Trk - isolated from a colon carcinoma
  - activated by fusion (with tropomyosin)
- Ret - isolated from a thyroid carcinomas
  - activated by fusion (with PTC1 and others)
- Oncogenic fusion proteins involving tyrosine kinases (PTC1-Ret):
  - the N-terminal sequences of the oncoprotein (Ret) are often replaced by the fusion partner (PCT1), resulting in deregulation of the kinase domain in the C-terminal half of the oncoprotein.
More proto-oncogenes

- identification of transforming DNA fragments in NIH3T3 transformation assays increased the number of known proto-oncogenes.

Oncogene complementation *in vitro*

- conduct focus formation assay using...
  - primary rat embryo fibroblasts (REFs)
  - these have not undergone “crisis”

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- transflect REFs with expression plasmids encoding...
  - ras* – c-H-ras with an oncogenic mutation at amino acid 12
  - myc – high levels of c-myc protein
Oncogene complementation *in vitro*

- results of focus formation assay…

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<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>(senescence)</td>
<td></td>
</tr>
<tr>
<td>myc</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>(apoptosis)</td>
<td>(apoptosis)</td>
</tr>
<tr>
<td>ras* + myc</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

→ ras* and myc cooperation is required for transformation of primary fibroblasts.

*in vivo* gene transfer experiments

- fibroblasts transformed *in vitro* with activated proto-oncogenes will induce tumors in nude mice.
  - can activated proto-oncogenes also induce *in vivo* tumors directly?
  - can activated proto-oncogenes induce *in vivo* tumors from cells of other (non-fibroblast) cell lineages?

- these questions can be addressed by developing transgenic animals that express activated proto-oncogenes
in vivo gene transfer experiments

- the MMTV-ras* transgene

```
+----------------+-------------------+
| MMTV            | c-H-ras (G12V)    |
| LTR             |                   |
+----------------+-------------------+
```

- encodes the activated c-H-ras gene from the EJ tumor line
  - with oncogenic missense mutation G12V
- transcriptional regulation of the transgene is mediated by the LTR element of the Mouse Mammary Tumor Virus (MMTV).
- the LTR of MMTV is tissue-specific, inducing transcription primarily in mammary epithelial cells.

MMTV-ras* transgenic mice

- female (but not male) MMTV-ras* transgenic mice develop mammary carcinomas.
  - about half the female transgenic mice form palpable mammary carcinomas in six months ($T_{50} \sim 6$ months).
  - the carcinomas are monoclonal (even though the transgene is expressed in all developing mammary epithelial cells).

$\rightarrow$ the latency and monoclonality indicate that additional somatic events are necessary for tumor formation from MMTV–ras* mammary cells.
MMTV-myc transgenic mice

- the MMTV-myc transgene
- female (but not male) MMTV-myc transgenic mice develop mammary carcinomas.
  - the T\textsubscript{50} of female transgenic mice is \sim 11 months.
  - the carcinomas are monoclonal

→ additional somatic events are also necessary for tumor formation from MMTV–myc mammary cells.

Oncogene complementation \textit{in vivo}

- mate MMTV-ras\textsuperscript{*} and MMTV-myc mice.
- female (but not male) \textbf{double} transgenic mice develop mammary carcinomas.
- much shorter latency (T\textsubscript{50} \sim 6 weeks).
  → cooperation between activated ras and myc gene \textit{in vivo}.
- again, the tumors are monoclonal
  → additional somatic events are also necessary for tumor formation from double-transgenic mammary cells.
Cytogenetics of normal cells

- traditional karyotype analysis
  - chromosomes condense during mitosis.
  - visible under the light microscope.
  - mitotic chromosomes can be stained with certain dyes.
  - for a given dye (e.g., Giemsa), each chromosome produces a unique and reproducible banding pattern.

- the karyotype of normal human diploid cells
  - one pair of sex chromosomes and 22 pairs of autosomes.
  - males: 46XY
  - females: 46XX

Cytogenetics of tumor cells - I

- karyotypes of tumor cells
  - tumor cells often have unstable chromosomes

- most tumor cells have chromosome abnormalities
  - abnormalities in chromosome number (aneuploidy)
  - abnormalities in chromosome structure
    - Translocations
    - Inversions
    - Deletions
    - DNA amplification (HSRs, DMs)
Cytogenetics of tumor cells - II

- most chromosome abnormalities of tumor cells appear to be random
  
  - they do not exhibit a particular pattern of association with specific tumors.

- however, careful studies revealed unique chromosome defects that recur in different patients with the same type of tumor.

- Do “recurrent” chromosome abnormalities reflect genetic lesions that promote tumorigenesis?
  
  - are cellular proto-oncogenes located near the newly recombined cytogenetic breakpoints malignantly activated?

Cytogenetics of tumor cells - III

- Carcinomas often have highly complex karyotypes with many, seemingly random, chromosome abnormalities.

- Hematopoietic tumors and sarcomas have less complex karyotypes. These often feature recurrent chromosome abnormalities that are characteristic of a particular type of cancer.
In 1960, Nowell observed an altered (shorter) form of chromosome 22 in many patients with chronic myelogenous leukemia (CML):

- Philadelphia chromosome (Ph\(^1\)) only found in the leukemic cells of CML patients
- Ph\(^1\) only associated with certain malignancies:
  - 95% of CML
  - 20% of adult acute lymphoblastic leukemias (ALL)
  - 5% of childhood ALL

→ Hypothesis: Ph\(^1\) represents a genetic lesion that can promote the formation of CML or ALL

The Philadelphia (Ph\(^1\)) chromosome

- Chromosome banding techniques revealed...
  - that Ph\(^1\) is the product of a reciprocal translocation between chromosomes 9 and 22:
    \[ t(9;22)(q34;q11) \]
  - t(9;22) has breakpoints at positions 9q34 and 22q11

→ Hypothesis: Ph\(^1\) promotes leukemia by malignantly activating a proto-oncogene located at 9q34 or 22q11

Ph\(^1\) produced by a chromosome translocation
Cytogenetics terminology

- the t(9;22)(q34;q11) chromosome translocation
  - translocation breakpoints occur at positions 9q34 and 22q11
  - 9 and 22 are the chromosomes.
  - chromosome short and long arms are “p” and “q”, respectively.
  - 9q34 is giemsa band 34 on long arm of chromosome 9
  - 22q11 is giemsa band 11 on long arm of chromosome 22

the t(9;22)(q34;q11) translocation

- reciprocal translocation
  - chromosomes 9 and 22
  - chromosome breakage at positions 9q34 and 22q11
  - reciprocal exchange of telomeric regions of 9 and 22
the t(9;22)(q34;q11) translocation

- the reciprocal translocation yields two derivative chromosomes:
  - the der(9) chromosome
    - has the centromere of chr. 9
  - the der(22) chromosome
    - has the centromere of chr. 22
    - same as Ph₁

- chromosome breakage
  - disrupts the BCR gene at 22q11
  - disrupts the ABL gene at 9q34
    - ABL is the cellular form of v-abl, a retrovirally-tranduced oncogene!!

- the der(22) junction has a ...
  - BCR-ABL fusion gene
The BCR-ABL fusion gene

- lies at the junction of der(22)
- contains:
  - transcriptional promoter of BCR
  - one or more 5’-coding exons of BCR
  - coding exons 2-12 of ABL

The BCR-ABL fusion protein

- RNA transcription of BCR-ABL fusion gene
- spliced BCR-ABL messenger RNA
- the BCR-ABL fusion polypeptide
Malignant activation of the ABL protein

- the normal ABL protein
  - a non-receptor protein tyrosine kinase
  - phosphorylation of ABL substrates promotes cell growth
  - enzymatic activity of normal ABL is tightly controlled

- the BCR-ABL fusion protein
  - retains the tyrosine kinase activity of ABL, but...
  - its enzymatic activity cannot be downregulated due to:
    - transcriptional deregulation by the BCR promoter
    - replacement of N-terminal ABL sequences with BCR sequences
    - the fusion protein constitutively promotes cell growth.

⇒ BCR-ABL is a malignantly activated form of the ABL proto-oncogene.

Animal models of BCR-ABL leukemia

- BCR-ABL retrovirus
  - infect murine hematopoietic stem cells \textit{in vitro}
  - use these stem cells to reconstitute lethally-irradiated mice
  - 50% of mice develop CML-like disease

- BCR-ABL transgene
  - produce mouse strain with a BCR-ABL transgene
  - mice develop acute leukemia within two months of birth

- The tyrosine kinase activity of BCR-ABL is essential for its transforming potential.
Clinical impact of Ph^1 chromosome

- diagnosis
- prognosis
- disease monitoring
- treatment

Burkitt’s lymphoma

- B cell malignancy
  - endemic in the malarial belt
  - common in immunosuppressed populations

- cytogenetic abnormalities associated with BL
  - ~ 90% of patients: t(8;14)(q24;q32)
  - ~ 5% “ “ “ t(2;8)(p12;q24)
  - ~ 5% “ “ “ t(8;22)(q24;q11)

- a common breakpoint at 8q24
Burkitt’s lymphoma II

- *in situ* chromosome hybridization of known oncogenes:
  - MYC gene located at 8q24.

- Southern analysis of genomic DNA from BL cells:
  - MYC gene rearranged in most cases of BL.

- Isolation of rearranged MYC fragment from BL cells:
  - Rearranged fragment encompasses the t(8;14) junction.

![Diagram showing MYC gene localization with 8q24 and 14q32 breakpoints](image)

Burkitt’s lymphoma III

- 14q32 break: immunoglobulin heavy chain gene (IgH) !

<table>
<thead>
<tr>
<th>translocation</th>
<th>break</th>
<th>locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;14)(q24;q32)</td>
<td>14q32</td>
<td>IgH</td>
</tr>
<tr>
<td>t(2;8)(p12;q24)</td>
<td>2p12</td>
<td>Igκ (kappa light chain gene)</td>
</tr>
<tr>
<td>t(8;22)(q24;q11)</td>
<td>22q11</td>
<td>Igλ (lambda light chain gene)</td>
</tr>
</tbody>
</table>

- MYC is malignantly activated upon juxtaposition with the any of the three immunoglobulin loci.

- This makes sense...
  - MYC activation by proviral integration causes B cell tumors in chickens congenitally infected with ALV.
  - The Ig genes undergo DNA recombination during normal B cell development
  - The Ig genes are transcriptionally active in normal B cells
Follicular lymphoma

- a distinct histopathologic type of B cell malignancy
- > 85% of cases have the t(14;18)(q32;q21)
  - 14q32 breaks in the IgH locus.
  - 18q21 breaks in the BCL2 gene.
  - a new proto-oncogene not encountered in animal studies?
- BCL2/IgH transgenic mice develop B cell tumors!

Mantle cell lymphoma

- another histopathologic type of B cell malignancy
- ~ 30% of cases have the t(11;14)(q13;q32)
  - 14q32 breaks in the IgH locus.
  - 11q13 breaks near the cyclin D1 gene (CCND1).
- cyclin D1 is a positive regulator of cell cycle progression that drives the G1/S transition by phosphorylating Rb
T cell acute lymphoblastic leukemia (T-ALL)

- clinically homogenous disease
- cytogenetically heterogeneous (in contrast with BL or FL)
  - over 12 different recurrent translocations seen in T-ALL
  - each found in a small proportion (<10%) of T-ALL patients
- T-ALL derived from immature T cells (thymocytes)
- The Ig genes serve as activating loci in translocations of B cell tumors. Thus, do the TCR genes serve a similar role in translocations of T cell tumors?
  - T cell receptor (TCR) β chain gene maps to 7q34
  - “ “ “ α/δ chain gene maps to 14q11

Recurrent translocations of T-ALL

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Patients</th>
<th>TCR</th>
<th>Oncogene</th>
</tr>
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<tbody>
<tr>
<td>t(8;14)(q24;q11)</td>
<td>~ 2%</td>
<td>α/δ</td>
<td>MYC</td>
</tr>
<tr>
<td>t(7;14)(q24;q11)</td>
<td>~ 2%</td>
<td>α/δ</td>
<td>LCK</td>
</tr>
<tr>
<td>t(7;9)(q34;q34)</td>
<td>~ 1%</td>
<td>β</td>
<td>NOTCH1</td>
</tr>
<tr>
<td>t(10;14)(q24;q11)</td>
<td>~ 3%</td>
<td>α/δ</td>
<td>HOX11</td>
</tr>
<tr>
<td>t(1;14)(p34;q11)</td>
<td>~ 3%</td>
<td>α/δ</td>
<td>TAL1</td>
</tr>
<tr>
<td>t(7;9)(q34;q32)</td>
<td>~ 2%</td>
<td>β</td>
<td>TAL2</td>
</tr>
<tr>
<td>t(7;19)(q34;p13)</td>
<td>&lt; 1%</td>
<td>β</td>
<td>LYL1</td>
</tr>
<tr>
<td>t(11;14)(p15;q11)</td>
<td>~ 1%</td>
<td>α/δ</td>
<td>LMO1</td>
</tr>
<tr>
<td>t(11;14)(p13;q11)</td>
<td>~ 5%</td>
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- all result in activation of an oncogene by recombination with the TCR locus at either 7q34 or 14q11
### Recurrent translocations of T-ALL

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- MYC activation by IgH in Burkitt’s lymphoma
- MYC activation by TCR in T-ALL
- MYC activated by retroviral transduction in T cell tumors of cats
- LCK activated by retroviral integration in mouse thymomas
### Recurrent translocations of T-ALL

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<td>~ 3%</td>
<td>α/δ</td>
<td>HOX11</td>
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- NOTCH1 was a novel proto-oncogene; its malignant potential was confirmed by tumor induction in transgenic mice
- NOTCH1 is malignantly activated by point mutations in almost 50% of T-ALL patients!!

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<td>α/δ</td>
<td>HOX11</td>
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- HOX11 was a novel proto-oncogene; its malignant potential was confirmed by tumor induction in transgenic mice
- HOX11 is malignantly activated by ectopic expression in ~25% of T-ALL patients!!
### Recurrent translocations of T-ALL

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<td>~2%</td>
<td>α/δ</td>
<td>LCK</td>
</tr>
<tr>
<td>t(7;9)(q34;q34)</td>
<td>~1%</td>
<td>β</td>
<td>NOTCH1</td>
</tr>
<tr>
<td>t(10;14)(q24;q11)</td>
<td>~3%</td>
<td>α/δ</td>
<td>HOX11</td>
</tr>
<tr>
<td>t(1;14)(p34;q11)</td>
<td>~3%</td>
<td>α/δ</td>
<td>TAL1</td>
</tr>
<tr>
<td>t(7;9)(q34;q32)</td>
<td>~2%</td>
<td>β</td>
<td>TAL2</td>
</tr>
<tr>
<td>t(7;19)(q34;p13)</td>
<td>&lt;1%</td>
<td>β</td>
<td>LYL1</td>
</tr>
</tbody>
</table>

- TAL1 was a novel proto-oncogene; its malignant potential was confirmed by tumor induction in transgenic mice.
- TAL1 is malignantly activated by gene rearrangement and ectopic expression in over 50% of T-ALL patients!!

- TAL1, TAL2, and LYL1 encode highly related proteins
- Presumably, activation of these genes represents an equivalent step in T cell leukemogenesis.
Recurrent translocations of T-ALL

<table>
<thead>
<tr>
<th>translocation</th>
<th>patients</th>
<th>TCR</th>
<th>oncogene</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;14)(q24;q11)</td>
<td>~ 2%</td>
<td>α/δ</td>
<td>MYC</td>
</tr>
<tr>
<td>t(7;14)(q24;q11)</td>
<td>~ 2%</td>
<td>α/δ</td>
<td>LCK</td>
</tr>
<tr>
<td>t(7;9)(q34;q34)</td>
<td>~ 1%</td>
<td>β</td>
<td>NOTCH1</td>
</tr>
<tr>
<td>t(10;14)(q24;q11)</td>
<td>~ 3%</td>
<td>α/δ</td>
<td>HOX11</td>
</tr>
<tr>
<td>t(1;14)(p34;q11)</td>
<td>~ 3%</td>
<td>α/δ</td>
<td>TAL1</td>
</tr>
<tr>
<td>t(7;9)(q34;q32)</td>
<td>~ 2%</td>
<td>β</td>
<td>TAL2</td>
</tr>
<tr>
<td>t(7;19)(q34;p13)</td>
<td>&lt; 1%</td>
<td>β</td>
<td>LYL1</td>
</tr>
<tr>
<td>t(11;14)(p15;q11)</td>
<td>~ 1%</td>
<td>α/δ</td>
<td>LMO1</td>
</tr>
<tr>
<td>t(11;14)(p13;q11)</td>
<td>~ 5%</td>
<td>α/δ</td>
<td>LMO2</td>
</tr>
</tbody>
</table>

- LMO1 and LMO2 encode highly related proteins
- LMO- and TAL-related proteins form stable nuclear complex; thus, a common pathway for T-ALL

Recurrent translocations of T-ALL

- Analysis of chromosome translocations identified three major pathways of T-cell leukemogenesis…
  - NOTCH1 pathway
  - HOX11, HOX11L2 pathway
  - TAL1, TAL2, LYL1, LMO1, LMO2 pathway
- Almost all T-ALL patients show malignant activation of at least one of these pathways
- Many T-ALL patients show activation of two or more of these pathways
Karyotypes of human sarcomas

- often display recurrent chromosome abnormalities
  - many of these engender fusions with the EWS gene

<table>
<thead>
<tr>
<th>chromosome translocation</th>
<th>tumor type</th>
<th>fusion protein</th>
<th>DNA-binding domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;22)(q24;q12)</td>
<td>Ewings sarcoma</td>
<td>EWS-FLI1</td>
<td>ETS family</td>
</tr>
<tr>
<td>t(21;22)(q22;q12)</td>
<td>Ewings sarcoma</td>
<td>EWS-ERG</td>
<td>ETS family</td>
</tr>
<tr>
<td>t(7;22)(p22;q12)</td>
<td>Ewings sarcoma</td>
<td>EWS-ETV1</td>
<td>ETS family</td>
</tr>
<tr>
<td>t(7;22)(q12;q12)</td>
<td>Ewings sarcoma</td>
<td>EWS-ETV4</td>
<td>ETS family</td>
</tr>
<tr>
<td>t(7;22)(q12;q12)</td>
<td>clear cell sarcoma</td>
<td>EWS-ATF1</td>
<td>bZIP family</td>
</tr>
<tr>
<td>t(11;22)(p13;q12)</td>
<td>desmo. sarcoma</td>
<td>EWS-WT1</td>
<td>Zn finger family</td>
</tr>
</tbody>
</table>

- each of the EWS partners harbors a DNA-binding domain
- each fused to the transcriptional activation domain of EWS

Recurrent chromosome abnormalities in human carcinoma (2002)?

- t(12;15)(p13;q25): encodes ETV6-NTRK3 fusion gene
  - ETV6 (TEL), a transcription factor of the “ETS” family
  - Neurotrophin-3 receptor, a TM protein tyrosine kinase

- t(12;15) in
  - congenital fibrosarcoma (1998)
  - cellular mesoblastic nephroma (1998)
  - acute myeloid leukemia (1999)

- 2002 – t(12;15) found in secretory breast cancer (SBC)
  - ETV6-NTRK3 transcripts observed in 12 of 13 SBC cases!
  - not observed in other types of breast carcinoma

- The first recurrent translocation in human carcinoma!!
Recurrent chromosome abnormalities in human carcinoma (2005)?

- Tomlins et al., Science 310: 644-648, 2005

- Recurrent gene fusions in prostate carcinomas
  - the TMPRSS2-ERG and TMPRSS2-ETV1 fusions retain…
    - non-coding exon 1 of androgen-inducible TMPRSS2 gene
    - coding sequences for the DNA binding domains of either ERG or ETV6, transcription factors of the “ETS” family
  - result in deregulated expression of truncated ETS proteins

- ERG and ETV gene fusions seen in 23 of 29 cases of prostate cancer!!

- more to come?

---

- In general, chromosome translocations activate proto-oncogenes by one of two mechanisms

  1. By generating an aberrant fusion gene comprised of exons from two genes located adjacent to the recombinated cytogenetic breakpoints

  2. By transcriptional deregulation of a proto-oncogene at one cytogenetic breakpoint upon juxtaposition with regulatory sequences from the other breakpoint
Gene amplification

- Gene amplification in response to metabolic stress
  - a common mechanism by which mammalian cells acquire resistance to certain metabolic inhibitors

- DHFR and methotrexate
  - DHFR (dihydrofolate reductase): an enzyme required for dNTP biosynthesis (and, in turn, DNA synthesis)
  - methotrexate: an analog of dihydrofolate that irreversibly binds and inhibits DHFR

```
  methotrexate
  ↓
DHFR  →  THF  →  →  dNTP synthesis
  DHF       THF
  ↓
DHF  →  DHFR  →  THF
```

Methotrexate-resistant cell lines

- **methotrexate** resistance
  - treat cells with methotrexate.
  - select for cells that grow in the presence of methotrexate.
  - methotrexate-resistant cells show gross amplification of the DHFR gene (and increased levels of DHFR protein)!!
  - DHFR gene amplification is manifested cytogenetically by the presence of...
    - DMs (double minutes)
    - HSRs (homogeneously staining regions)
DMs and HSRs

- double minute (DMs) chromosomes
  - small, independent, chromosome-like structures
  - comprised of multiple tandem “amplicons” of genomic DNA that harbor the selected gene (e.g., DHFR)
  - lack centromeres
  - genetically unstable

- homogenously-staining regions (HSRs)
  - multiple tandem amplicons that harbor the selected gene (e.g., DHFR) incorporated into a chromosome
  - stain homogenously (i.e., lack the banding pattern of normal chromosomal material)
  - derived by integration of DM sequences into a chromosome
  - genetically stable

Gene amplification in human tumors

- neuroblastomas
  - DMs and HSRs are very common in human neuroblastomas
  - correlate with a poor prognosis
  - the amplicons of these structures often cross-hybridize with sequences from the c-Myc gene

- amplification of the MYC gene family in tumors
  - c-Myc: neuroblastomas
  - N-Myc: neuroblastomas
  - L-Myc: lung carcinomas

- amplification of the MDM2 gene in human sarcomas
Proteins encoded by proto-oncogenes

- Many proto-oncogenes encode components of signal transduction pathways that promote cell proliferation in response to...
  - internal cues
  - extracellular stimuli

- Growth factors
- Growth factor receptors
- Cytoplasmic transducers
- Nuclear factors

Growth factors and their receptors

- Growth factors
  - Simian Sarcoma Virus harbors a transduced oncogene (v-sis)
  - The c-sis gene encodes B chain of platelet-derived growth factor (PDGF)
  - Mechanism: autocrine stimulation of cell growth

- Growth factor receptors
  Transmembrane protein tyrosine kinases
  - c-erbB encodes the epidermal growth factor (EGF) receptor.
  - c-fms encodes the CSF-1 receptor.
  - Mechanism: constitutive activation of a receptor in the absence of its cognate ligand.
Figure 5.11c The Biology of Cancer (© Garland Science 2014)

Figure 5.11a The Biology of Cancer (© Garland Science 2014)
Cytoplasmic transducers

- Protein tyrosine kinases: c-src, c-fes, c-abl
- Protein serine/theonine kinases: c-raf and c-mos
- the Ras proteins (small G proteins)
  - bind the guanine nucleotides GDP and GTP
  - have GTPase activity: convert GTP to GDP
  - have a covalently-attached farnesyl group that inserts into the inner leaflet of the cell membrane
  - GTP-bound Ras proteins stimulate downstream pathways (including the Raf/MAP kinase cascade)
  - Oncogenic mutations ablate the GTPase activity. Thus, mutant Ras proteins constitutively stimulate downstream pathways.
Nuclear factors

- Transcription factors activated by signaling pathways
  - c-myc
  - c-jun
  - c-fos

Other categories of proto-oncogenes

- Negative regulators of the tumor suppressor pathways
  - MDM2 (amplified in human sarcomas) is a feedback inhibitor of the p53 tumor suppressor.
  - Cyclin D1 (translocated in centrocytic B cell lymphomas) associates with CDK4/6 to form kinase complexes that phosphorylate Rb and inhibit its tumor suppression activity.

- Inhibitors of apoptosis
  - BCL2 (translocated in follicular B cell lymphomas) encodes an anti-apoptotic protein.
  - E2A-HLF, a fusion gene associated with pre-B cell acute lymphoblastic leukemia, encodes a transcription factor that suppresses apoptosis.
Clinical impact of Ph¹ chromosome

- diagnosis
- prognosis
- disease monitoring
- treatment

Diagnosis & Prognosis

- diagnosis
  - The presence of Ph¹ in granulocytic cells of PB or BM is tantamount to a diagnosis of CML

- prognosis
  - Ph¹-positive ALL has an especially poor outcome in both children (~5%) and adults (~20%)

- disease monitoring
- treatment
CML progression

- chronic (pre-malignant) phase
  - leukocytosis and circulating immature granulocytic cells
  - chronic phase lasts ~5 years
  - Ph<sup>1</sup> translocation occurs in a hematopoietic progenitor cell
  - Ph<sup>1</sup> cells differentiate normally

- accelerated phase
  - additional genetic lesions?
  - rising numbers of Ph<sup>1</sup> blasts and basophils in PB or BM
  - lasts 6–18 months

- blast crisis
  - acute myeloid or lymphoid leukemia
  - lasts 3–6 months

Disease Monitoring

- to measure the levels of Ph<sup>1</sup>-positive cells during…
  - the chronic phase and accelerating phase
  - response to chemotherapy or transplantation

- Ph<sup>1</sup>-positive cells detected by…
  - cytogenetic analysis of metaphase spreads
  - fluorescent in situ chromosome hybridization (FISH) of interphase cells
  - quantitative PCR (or RT-PCR) of patient DNA (or RNA) using primers that flank the translocation junction.
conventional CML treatment (before Gleevac)

■ chronic phase
  ● hydroxyurea (RNR inhibitor)
    ♦ for cytoreductive therapy
    ♦ does not significantly alter disease progression
  ● interferon α
  ● allogeneic stem cell transplantation
    ♦ only proven curative therapy
    ♦ not an option for most CML patients

■ blast crisis
  ● acute myeloid leukemia
    ♦ standard induction chemotherapy: 20% response
    ♦ complete remission: < 10%
  ● acute lymphoid leukemia
    ♦ standard induction chemotherapy: 50% response
    ♦ complete remission: < 10%

Gleevac (imatinib mesylate or STI571)

■ the deregulated enzymatic (tyrosine kinase) activity of BCR-ABL is the essential transforming event in CML

  ● Ciba-Geigy (Novartis) identified a synthetic tyrosine kinase inhibitor (2-phenolaminopyrimidine)
  ● blocks the ATP-binding site of tyrosine kinases
  ● screened compounds for increased potency and specificity
  ● Gleevac: specific inhibitor of …
    ♦ ABL
    ♦ c-Kit
    ♦ PDGF-Rβ
cellular effects of Gleevac

- inhibits proliferation of cell lines containing BCR-ABL
- inhibits clonal growth of Ph^1^ cells from CML patients
- inhibits the *in vivo* growth of BCR-ABL-expressing cells in animal models of CML

Clinical study of Gleevac

- Phase 1, dose-escalating trial (NEJM 344:1031, 2001)
- chronic phase CML patients for whom interferon α treatment had failed
- daily doses ranging from 25 to 1000 mg
- all doses tolerated very well
- low doses (25, 50, 85 mg): half of patients removed from study within 2 mos. due to increasing WBC counts
- high doses (300-1000 mg): 53 of 54 patients showed a complete hematologic response within a month
  - maintained for 310 days in 51 patients