Cytogenetics, Gene Fusions, and Cancer*†‡

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ABSTRACT

Cytogeneticists recognize that karyotypic abnormalities are associated with specific malignancies. In 1960, Nowell described the Philadelphia chromosome (Ph) and its relationship to chronic myelogenous leukemia (CML). Subsequent work in molecular genetics and biology has revealed that the Ph is a translocation that causes fusion of gene sites that code for the break cluster region (BCR) and the avian blastic leukemia (ABL) proteins. This so-called fusion protein is present in a large percentage of the patients who have CML. A related fusion protein is seen in about one third of patients with acute lymphoblastic leukemia. The BCR-ABL fusion protein results in increased tyrosine kinase activity. The mechanism of action is thought to be via signal transduction related to guanosine triphosphatase activating protein which interacts with a ras-p21 binding protein.

Acute promyelocytic leukemia (APL) is associated with the cytogenetic abnormality of t(15;17). This alters the promyelocytic leukemia (PML) and the retinoic acid receptor α (RARA) gene sites. Two fusion proteins are the result of this cytogenetic abnormality. They are termed PML-RARA and RARA-PML. Only one, the PML-RARA, is associated with APL. The PML-RARA chimeric protein has two zinc finger-like regions. It retains the ligand binding domain of RARA. The protein called PML has some similarities with a family of proteins which are thought to fuse to proto-oncogenes and to act as transforming proteins. The role of classical cytogenetics and the added capability of molecular biology has helped to elucidate some of the potential mechanisms for the development of cancer and provided additional understanding of neoplasia. Whether or not these techniques will be useful in earlier diagnosis, prevention, or possibly the routes for successful therapies, are yet to be demonstrated.

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Introduction

The first chromosomal abnormality associated with cancer was described by Nowell and Hungerford in 1960. This change was noted before the time of chromosomal banding and was recognized only as a shortened portion of chromosome 22. The place of discovery was Philadelphia, Pennsylvania, and, hence, this altered chromosome has been called the Philadelphia chromosome (Ph). Advances in cytogenetic banding techniques occurred in the late 1960s and early 1970s. This provided the opportunity for identification of additional specific chromosomal rearrangements. It was not until 1973 that Rowley identified the reciprocal translocation between chromosomes 9 and 22 as an important part of the Philadelphia chromosome chronic myelogenous leukemia (CML) relationship. Subsequent molecular techniques have revealed that this translocation involves the c-abl oncogene which is present at the 9q34 and the breakpoint cluster region (bcr) which is on chromosome 22 at q11. This hybrid gene produces a fusion protein which results in increased tyrosine kinase activity which in turn leads to unchecked growth and malignant myeloid cell transformation. Several additional fusion proteins related to myeloproliferative disorders have been recognized and characterized.

Objective

The object of this work is to review and explore the role of cytogenetics as an aide in understanding fusion proteins and the pathogenesis of myeloproliferative disorders.

Methods

Cytogenetic translocations and fusion proteins for specific myeloproliferative disorders are evaluated. These include t(9;22) (q34;q11), t(8;21) (q22;q22), t(15;17) (q22;q12), t(11;17) (q23;q21) and inv(16) (p13;q22) described originally by karyotyping (Table I). Additional studies included fluorescent in-situ hybridization, polymerase chain reactions and where applicable Western blot techniques.

Results and Discussion

The classical cytogenetic translocation associated with Ph is t(9;22) (q34;q11). This results in a fusion protein of 210kd molecular weight known as p210. Nowell described the shortened small chromosome related to chronic myelogenous leukemia in 1960. It was not until 1973, after banding techniques were available, that the true nature of the disorder relating changes to two chromosomes (9 and

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Fusion Protein</th>
<th>Disease</th>
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<tbody>
<tr>
<td>t(9;22) (q34;q11)</td>
<td>BCR-ABL</td>
<td>CML</td>
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<td>t(8;21) (q22;q22)</td>
<td>AML-ETO</td>
<td>AML-M2</td>
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<tr>
<td>t(15;17) (q22;q12)</td>
<td>PML-RARα</td>
<td>APL</td>
</tr>
<tr>
<td>t(11;17) (q23;q21)</td>
<td>PLZF-RARα</td>
<td>Atypical APL</td>
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<tr>
<td>inv(16) (p13;q22)</td>
<td>CBFβ-MYH 11</td>
<td>AML M4 Eo</td>
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</tbody>
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BCL = breakpoint cluster region.
ABL = avian blastic leukemia.
AML = acute myelogenous leukemia.
ETO = eight twenty one.
CML = chronic myelogenous leukemia.
PML = promyelocytic leukemia.
RARα = retinoic acid receptor alpha.
PLFZ = promyelocytic leukemia zinc finger.
APL = acute promyelocytic leukemia.
CBFβ = core binding protein factor beta.
MYH = myosin heavy chain protein.
M4 Eo = AML type M4 with eosinophils.
22) was clearly identified. Review of 138 recent patients at the U.T. M.D. Anderson Cancer Center with CML or CML-like clinical presentations revealed that 96% of these patients had the aforementioned translocation or a recognized variant. Indeed, the definition of CML has come to be linked to the presence of the recognized translocation. The explanation for the association of t(9;22) with CML is that the abl site of chromosome 9 is related to tyrosine kinase activity. The bcr site on chromosome 22 is related to guanosine triphosphatase (GTPase) activating protein which is a ras-related GTP binding protein. This combination apparently causes up-regulation of tyrosine kinase and GTPase activity. The result is deregulation of myeloid differentiation with the resultant malignant transformation of the granulocytic cell line.

Of note is the fact that a bcr-abl fusion protein is also seen in a percentage of patients with acute lymphocytic leukemia (ALL). There is a slightly different break in the region of the bcr and this results in a fusion protein identified as p185. The p185 in ALL is associated with a worse prognosis than the bcr-abl p210 fusion protein of CML.

As early as 1982, a translocation seen in 8 to 18 percent of patients with AML of the FAB Type M2 was described. This translocation known as t(8;21) (q22;q22) results in a fusion protein of the AML 1 gene product from chromosome 21 and the eight twenty one (ETO) gene product from chromosome 8. This leukemia is characterized by the prominent presence of Auer rods, basophilic cytoplasm of the myeloblasts, and splenic myeloblastomas. The M2 leukemias with t(8;21) usually involve a younger patient population and have a better survival prognosis. The favorable prognosis is secondary to higher remission rates after aggressive therapy. The AML-1 gene has significant homology to the "runt" gene of Drosophila. This gene is responsible for segmentation in the fruit fly. Translocation of AML-1 and ETO apparently results in activation of ETO gene expression and subsequent inability for myeloid differentiation to occur.

Acute promyelocytic leukemia (APL), classified in the French, American, British (FAB) nomenclature as M3 was first recognized to be associated with a characteristic translocation in 1977 by Okada et al and described further by Borrow, et al and de Thé, et al. This translocation was noted to be p(15;17) (q22;q21). Chang et al in 1992 described the molecular biological result of this translocation. Two fusion proteins are found. One is the promyelocytic leukemia-retinoic acid receptor alpha (PML-RARα) fusion protein which is associated with the production of APL. The other reverse translocation of retinoic acid receptor alpha-PML fusion protein is not associated with the same disease characteristics. This PML activity is disrupted as a result of the t(15;17) alteration. The PML gene suppresses growth rate, alters clonogenicity and tumorigenicity of certain cells under experimental conditions. Promyelocytic leukemia gene is also noted to induce apoptosis. The result of the disruption of normal PML activity when it is bound to the RARα is that there is unchecked growth and leukemogenesis. Promyelocytic leukemia allele is a zinc finger transcription factor gene. The presence of the retinoic acid receptor alpha has led to the use of all transretinoic acid (ATRA) as a life-saving therapy in patients presenting with APL.

A translocation associated with an APL-like clinical condition was described in 1993 and 1994. This t(11;17) (q23;q21) has a morphology which is intermediate between M2 and M3. It is the result of a fusion of a promyelocytic leukemia zinc finger (PLZF) and the retinoic acid receptor alpha (RARα) gene sites. The PLZF is a member of the cystine and histidine containing zinc fin-
ger transcription factors. It is localized to the nucleus but generally disbursed throughout the nucleus. The mechanism of action of PLZF is DNA binding and is a transcriptional effector. The PLZF apparently regulates genes that are required for white blood cell development. The patients with the t(11;17) abnormality do not respond to all-trans-retinoic acid therapy. Their course and prognosis are generally worse than that of t(15;17) APL.\textsuperscript{13,14}

A unique inversion which has the same characteristics as translocation is seen with AML, M4Eo.\textsuperscript{15} This cytogenetic abnormality described as inv16 (p13;q22) is also seen as a translocation noted as t(16;16) (p13;q22). The two genes involved are core binding protein factor beta (CBF\textbeta) which is also known as (PEBF2\textbeta) and the myosin heavy chain protein (MYH11) site. In vitro, CBF\textbeta binds with CBF alpha in a heterodimer and then interacts with DNA. The CBF\textalpha is identical to the AML-1 gene noted in the t(8;21). The CBF\textbeta/MYH11 fusion protein is associated with the presence of increased eosinophils in AML-M4. AML-M4 with increased eosinophils has a relatively favorable prognosis when compared to standard M4. Only 10% of AML M4s have this translocation.

**Summary**

Cytogenetic abnormalities recognized by classical karyotyping which are related to hematologic malignancies were first reported in 1960 and continue to be described today. Review of the translocations noted in CML, M2, M3, and M4Eo reveals that each of them is associated with a specific fusion protein which results in the increase of cellular intermediary metabolism changes often through a kinase. There is a resultant inability of the myeloid cell line to hold growth in check or to mature or differentiate. Specific cytogenetic abnormalities with clearly identified breakpoints result in fusion proteins which have unique identities and activities. Fusion proteins are noted in several of the myeloproliferative disorders and in some of the lymphoproliferative disorders. Cytogenetics and these fusion proteins can be used for diagnosis, prognosis and monitoring of the therapeutic response. The cytogenetic and molecular diagnostic techniques are most effective when used in a complementary approach. Sites of translocation and fusion proteins may be targets for future genetic therapy or chemotherapeutic manipulation.

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**References**

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