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NEOPLASIA

Comment on Krejci et al, page 2190

New role for *AML1/ETO* in leukemogenesis

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The AML1/ETO fusion protein induces DNA double-strand breaks, p53 expression, and a DNA damage response, suggesting a new role in AML pathogenesis.

In this issue of *Blood*, Krejci and colleagues suggest that the transcription factor fusion protein, *AML1/ETO* (also known as *RUNX1/MTG8*), which is known to block differentiation in myeloid cells, may also increase DNA damage, adding to its functions in leukemogenesis. The *AML1/ETO* fusion protein was originally identified 15 years ago as the protein product of the t(8;21) translocation associated with M2 acute myeloid leukemia (AML).[1] The protein acts as a transcriptional regulator by recruitment of nuclear corepressors leading to inhibition of the core binding factor complex, which includes the *AML1* protein.[2] This inhibition leads to a block in myeloid differentiation, but in vivo, *AML1/ETO* expression is not sufficient to generate acute leukemia.[3] In their article, Krejci and colleagues study the expression of *AML1/ETO* in primary human CD34 cells. They demonstrate that cells expressing *AML1/ETO* induce expression of a DNA damage response that is p53 dependent, and suggest that the induction of a p53 response is associated with improved prognosis of t(8;21)$^+$ AML.

The authors use retroviral constructs to express *AML1/ETO* in primary human CD34$^+$ cells. As they have previously demonstrated,[4] expression of *AML1/ETO* "immortalizes" these cells so that they can be studied over the course of several weeks in culture. Consistent with previous results,[5] they make the observation that *AML1/ETO* expression leads to transcriptional down-regulation of genes involved in base excision repair of DNA damage, particularly the genes 8-oxoguanine glycosylase gene (*OGG1*), and polymerase epsilon gene (*POLE*).[5] Importantly, they demonstrate that binding of *AML1/ETO* is through a *RUNX1* binding site, suggesting that regulation of DNA damage-response genes may be a physiologic function of *RUNX1*. Curiously, although base excision repair is primarily involved in the repair of point mutations in DNA, the authors then demonstrate that *AML1/ETO*-expressing cells have an increase in DNA double-strand breaks and an up-regulation of p53 expression. Down-regulation of p53 expression with a siRNA approach in *AML1/ETO*-expressing cells makes the cells more sensitive to ionizing radiation and other DNA-damaging agents. Overall, the study makes a convincing argument that *AML1/ETO* expression leads to an altered response to spontaneous and induced DNA damage.

How are we to interpret these data in the context of a growing body of data about the multiplicity of functions of *AML1/ETO* and data about the role of DNA damage responses in hematologic malignancies? The answer to this is unknown, but the data do not support the idea that oncogenes such as *AML1/ETO* are simple stimulators of cell growth. If these complex proteins lead to development of a "super cell," they do so by inducing the cells to develop mechanisms to survive stress (a transforming boot camp of sorts). Expression of *AML1/ETO* actually induces apoptosis in some cells.[6] The presence of DNA double-strand breaks in these cells may represent the induction of replication stress and DNA breaks during S phase. The data leave unanswered the question of the necessity of this DNA damage-induced stress response for cellular transformation. However, the well-described structure of the *AML1/ETO* fusion may allow for structure-function studies that will better define the necessities and sufficiencies for transformation induced by this fascinating oncoprotein.

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CLINICAL OBSERVATIONS

Comment on Plug et al, page 1811

Hemophilia lives: the impact of prophylaxis

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High-cost prophylaxis for hemophilia has decreased morbidity, but its long-term impact on quality of life needs expanded investigation. This Arbeit takes a hard look at this and underscores its additional effects on the lives of these patients.

In this issue of *Blood*, Plug and colleagues in the Netherlands use their ongoing, country-wide, epidemiologic hemophilia studies to evaluate the effect of the introduction of prophylaxis on patients' ability to work and complete an education, as compared with the general population. More than 721 patients were divided into 2 groups: patients from 16 to 31 years of age after prophylaxis was introduced, and those from 31 to 64 years of age before its introduction.

As shown through the findings of this group and others, prophylaxis decreases disabilities. Of the hemophilia patients tested, 36% versus 5% of those with severe hemophilia had occupational disability before versus after introduction of prophylaxis. The study demonstrates that this health-care innovation not only affects joint outcomes, but also allows patients to achieve a higher level of education and career, such as by holding academic or managerial positions.

Health-related quality of life tools designed specifically for hemophilia are evolving. Plug and colleagues used a well-known scale, Short-Form 36, to evaluate their patient population. Patients with mild and moderate hemophilia were similar to the general population in both groups. In all 8 categories of measurement, there was marked improvement once prophylactic treatment was introduced.

These data strongly suggest that social integration into society is correlated, in the main, with the disabling sequelae of this disease. What is not addressed is the impact of transfusion-transmitted diseases on the quality of life, disability, and educational and/or work level. By using only questionnaires, one cannot evaluate the impact of the family structure, nor of socioeconomics, on issues such as educational expectations, academic achievements, and occupation. The authors do not comment on aspects of life not covered by the instrument they used. How did the change in therapy affect self-esteem, or patients' satisfaction with their life, family, and work?

The costs of prophylaxis are significantly higher than those of on-demand therapy.[1] The findings of Plug and colleagues, obtained in the Holland health-care system in which these costs are covered, may not be easily extrapolated to the United States, which has a pluralistic health-care reimbursement system and high costs of higher education. The message delivered by the authors is clear, nevertheless: their findings should inspire expansion of prophylaxis. The quality-of-life data they have presented will encourage the development of instruments to better understand the outcomes of the health-care delivery system, but translating them into meaningful financial paybacks to society will require further work.

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HEMATOPOIESIS

Comment on Freson et al, page 1885

PACAP: a new player in thrombopoiesis

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Freson and colleagues have identified a role (see figure) for the megakaryocyte VPAC1 receptor and its 2 major agonists, PACAP and VIP, in the regulation of platelet production.

The complex mechanisms underlying megakaryopoiesis and platelet biogenesis are poorly understood, and identification of new molecules associated with these processes is highly welcome and often opens multiple new lines of investigation. This likely will be the case for the "new kid on the block" reported in this issue of *Blood* by Freson and colleagues: PACAP (pituitary adenylyl cyclase–activating peptide).

PACAP shares a high degree of homology with vasointestinal peptide (VIP), which was suggested nearly 20 years ago to play a negative role in platelet aggregation.[1] Both PACAP and VIP show high affinity for the VIP receptor, VPAC1, which is expressed in both platelets and megakaryocytes.[2]

Clinically, it is known that trisomy of chromosome 18p results in platelet dysfunction and mild thrombocytopenia. Because PACAP is encoded on chromosome 18p, Freson and colleagues hypothesized that increased levels of this molecule (resulting from 3 copies of the *PACAP* gene) are responsible for the platelet dysfunction and thrombocytopenia observed in this syndrome. In another study, these same investigators demonstrated that megakaryocyte-specific transgenic overexpression of PACAP in mice led to decreased platelet activation.[3]

Freson and colleagues are extending their previous findings by providing evidence for the importance of the PACAP/VIP/VPAC1 axis in the regulation of platelet production. First, megakaryocytes from both patients and mice with excess copies of the PACAP gene were shown to exhibit signs of maturation arrest. Second, these investigators were able to stimulate megakaryopoiesis both in vitro and in vivo by inhibiting VPAC1 signaling with specific blocking antibodies. These observations demonstrate a clear negative regulatory role for PACAP in thrombopoiesis, which is likely secondary to decreased cAMP levels mediated by activation of VPAC1.

Perhaps an even more exciting component of this study is its investigation of potential therapeutic effects of inhibition of the PACAP/VIP/VPAC1 pathway. Using mice with congenital thrombocytopenia and rabbits with acquired thrombocytopenia, the authors demonstrate that infusion of a VPAC1-blocking antibody results in a significant (but temporary) elevation of the platelet count in a thrombopoietin-independent manner. Surprisingly, despite the potential pleiotropic effects of VPAC1 inhibition (VPAC1 is expressed in many other tissues including cells of the central nervous, gastrointestinal, and reproductive systems), no major adverse effects were observed.

Although this article successfully addresses the importance of the PACAP/VIP/VPAC1 axis, several mechanistic issues remain unclear. These include the downstream effects of VPAC1 inhibition that lead to raised platelet counts, the exact stages of platelet production in which VPAC1 is critical, and the potential consequences of VPAC1 inhibition in other systems if it is targeted for the treatment of