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Fanconi anemia: A paradigm of discovering molecular pathways from patients

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We are delighted to bring you this special edition on Fanconi anemia (FA), a rare disease that is characterized by bone marrow failure and a predisposition to cancer. Since FA is caused by an inherited defect in a genome maintenance pathway, it is particularly fitting for *Mutation Research* to sponsor this edition. As the title of this special edition indicates, FA is a classic example of a puzzling human disease that stimulates basic research, which leads to the discovery of a novel pathway for responding to cellular stress. This is a testimony to the power of clinical observation and the profound impact of scientific enquiry. We are therefore particularly delighted to include as authors in this special edition clinicians who treat FA patients and basic scientists who use various model organisms to do elegant genetic and interventional experiments to dissect the molecular basis of FA. This exemplifies how synergy between scientists with diverse talents can lead to exponential gains. There has been an explosive growth in knowledge over the last decade that has identified numerous FA genes, generated insight into the molecular function of the proteins encoded by FA genes, and led to an understanding of how these proteins are organized into a pathway that responds to replication-blocking lesions. As a result, the field is poised to provide improved therapeutic options for FA patients and novel strategies for treating cancer in the general population.

FA is characterized by chromosome instability and hypersensitivity to genotoxic stress, in particular that caused by DNA interstrand crosslinks (ICLs). This affects embryonic development, post-natal growth, and stem cell number and function, and results in a greatly elevated cancer risk in FA patients. Together, these phenotypes dramatically illustrate the vital importance of genome maintenance mechanisms. There are currently 13 identified FA genes (FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M and -N). Whether FANCM is actually a FA gene is in question, however, since FANCA is also mutated in cells from the FA patient in which FANCM mutations were initially identified [1]. Patients with biallelic mutation in any one of these genes have a clinically similar disease, suggesting that all of the

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proteins function in the same genetic pathway. However, the symptoms in FA are extremely heterogeneous, even between monozygotic twins. The multigenic nature of FA and this clinical heterogeneity present unique challenges to clinical diagnosis of the disease, to understanding its pathophysiology, and to the development of treatment strategies.

Interestingly, three of the FA genes – FANCF/BRIP1, FANCD1/BRCA2, and FANCN/PALB2 – are also breast cancer susceptibility genes (BRCA) in the general population. Further, other FA genes are mutated or silenced in various cancers in those without FA. Together, this suggests that investigation of the function of FA proteins will yield general insights into the genesis of cancer. This issue highlights the progress in understanding the FA-BRCA pathway at the level of protein function, protein:protein interactions, DNA metabolism, cell signaling, and pathophysiology.

The first paper in this issue, authored by Dr. Auerbach, provides a clinical introduction to FA and its diagnosis. The review eloquently describes the highly variable symptoms associated with FA, and as a consequence, the difficulties associated with patient diagnosis. Dr. Auerbach makes several critical observations based on her experience and review of the literature: (1) the published case reports of FA are biased towards very severe cases, leading to a biased view of the clinical manifestations of FA and contributing to the ongoing under-diagnosis of mild cases; (2) the tremendous clinical heterogeneity in FA provides strong evidence that the FA pathway is not part of a genetically regulated program of development, but in fact exists to protect against stochastic damage influenced at least in part by environmental factors.

The second review, authored by Drs. de Winter and Joenje, gives an historical look at the identification of FA genes and also compares different strategies for the identification of FA genes. These strategies include cloning by the complementation of hypersensitivity to DNA interstrand crosslinking agents, positional cloning, and the identification of proteins that interact with FA proteins in complexes. This review also gives an insightful summary of what is known about the function of individual FA proteins. Finally, on this basis, the authors provide a model for how FA proteins may function in a coordinated fashion to respond to replication stress.

The FA genes that were identified first – including FANCC, FANCA, and FANCG – did not encode proteins with functional motifs that yielded insight into their function. More recently, FANCF and FANCM have been identified as FA proteins that contain helicase domains. Further, FANCM contains a degenerate endonuclease domain. Thus, FANCF and FANCM may have enzymatic activities that are critical to the function of the FA-BRCA pathway. Drs. Ali, Singh, and Meetei review various structure–function and biochemical studies of FANCF and FANCM that have already generated important insights into how FA proteins may act to repair ICLs at the replication fork. While FANCF appears to unwind DNA structures that impede the replication fork, FANCM appears to have a role in sensing replication stress and in the recruitment of the FA core complex.

This is followed by a review by Dr. Howlett and associates that examines how characterization of the interactions of FA proteins with non-FA proteins has advanced our

understanding of FA protein function. This review considers the association of FA proteins with RAD51 and BRCA1, ATM and ATR, BLM, PCNA, and TIP60, which function in homologous recombination (HR), signaling of DNA damage responses, suppression of illegitimate recombination, translesion synthesis (TLS), and chromatin remodeling, respectively. These interactions underscore the role of FA proteins at the crossroads of different DNA repair pathways. Of particular interest, the significance of ATR-dependent phosphorylation of various FA proteins is considered. Also, the authors note that the association of the FA core complex with BLM led to the identification of FANCB, FANCL, and FANCM.

This is followed by a detailed review by Drs. Pang and Andreassen that delves into the types of DNA damage to which the FA-BRCA pathway responds and which therefore are potentially implicated in causing the symptoms associated with FA. They describe the endogenous sources of stress that are most likely to drive pathogenesis in FA, including oxidative DNA damage, telomere shortening, and replication stress. The review makes the important point that although FA is diagnosed by an aberrant response of FA cells to DNA crosslinking agents, it is likely that the FA pathway did not evolve to combat damage induced by these exogenous drugs. This has led to great controversy in the field as to the cause of the diverse set of pathologies associated with FA.

In their review, Drs. Thompson and Hinz take a step back and consider the large body of literature on cellular phenotypes of FA cells and vertebrate model systems for FA. This comprehensive review argues that FA proteins have functions beyond simply dealing with ICLs. Importantly, evidence is considered which sheds light on whether FA proteins function in error-prone or error-free repair, or both. Other phenotypes, including defective repair of ICLs or DNA double-strand breaks, and cell cycle checkpoint defects, are also critical for understanding the function of specific FA proteins. There are a myriad of discrepancies in the literature concerning the phenotypes of cells that are deficient for FA proteins. This review considers the basis for these discrepancies and culminates in an elaborate model of how FA proteins function at the replication fork. This model should be informative for future studies.

This is followed by a review authored by Drs. Neveling, Hoehn, and Schindler about how failure to respond to genotoxic stress affects FA patients. The review provides a thorough and quite unique analysis of how defects in the FA pathway lead to a widely diverse set of symptoms, even though they arise from a common cellular defect. The authors also examine whether various FA complementation groups and specific mutations in FA genes predict patient phenotype. The authors reach the novel conclusion that FA represents a model of segmental (or tissue-specific) accelerated aging. This is a recent finding in many genome instability disorders and is therefore extremely timely.

There is then a series of reviews that examine the utility and contributions of various model organisms to the understanding of FA. Dr. Takata and colleagues highlight the contributions of DT40 cells. This paper provides a comprehensive review of the substantial contributions made to our current understanding of the FA-BRCA pathway of DNA damage signaling by research done in chicken lymphocytes. DT40 cells are an excellent model system for

knocking-out genes. This has proved crucial for establishing epistasis between FA and other genome maintenance genes. Furthermore, it is straightforward to knock-in mutations in DT40 cells, which has enabled the analysis of the functions of FA proteins and has yielded tremendous insight into the biochemical mechanism of FA signaling.

Drs. Youds, Barber, and Boulton address the use of the nematode *Caenorhabditis elegans* (*C. elegans*) as a model organism for studying the function of FA proteins in the maintenance of genomic stability. This paper gives an excellent overview of the advantages of this system that is readily understandable to researchers that work with other models. Importantly, like DT40 cells, *C. elegans* can be used to determine epistasis. For example, work in *C. elegans* suggests that FANCD2 and FANCI are epistatic in ICL repair. Also, a novel role for FANCI in maintaining the stability of G-quadruplex DNA was first suggested by work in *C. elegans*. Interestingly, while the FANCD2 homolog of *C. elegans* can be monoubiquitinated, homologs of the FA core complex proteins have not been identified. Thus *C. elegans* could have a pared-down FA-BRCA pathway that might yield critical insights in the future.

Another model system, the zebrafish, is highlighted in a paper by Drs. Titus, Postlethwait, and colleagues. This review summarizes evidence that all 13 identified FA genes are present and that zebrafish therefore possess an FA network directly comparable to humans. Given the utility of zebrafish for studying developmental biology, it is an excellent model for understanding how the many congenital anomalies which are associated with FA, including microphthalmia, microcephaly, and forelimb defects, may arise during development. The authors provide novel data which suggest that FA proteins are highly expressed in rapidly dividing tissues that are critical to the development of organs affected in FA. Intriguingly, there are differences in the expression patterns of the various FA genes that may be the basis for understanding common and divergent functions of FA proteins and FA patient phenotypes.

Mouse models for FA are reviewed in an insightful paper by Drs. Parmar, D'Andrea, and Niedernhofer. Mice with mutation or deletion of five of the FA genes have been created. While *Fancc*^{-/-}, *Fancc*^{-/-}, and *Fancg*^{-/-} mice do not display severe hematological abnormalities or aplastic anemia, as is common in FA patients, these models have been extremely useful for studying stem cell failure and therapy. Importantly, mice which harbor a biallelic mutation of BRCA2 in its C-terminus or a conditional knockout for FANCD2, do display significantly compromised function of hematopoietic cells, increasing the utility of mouse models for FA. Also, genetic studies with FA mouse models (e.g., double mutant animals) are reviewed which have yielded novel insights into how FA genes function in hematopoiesis and as tumor suppressors.

One model organism not specifically covered in this issue, in which great strides have been made, is *Xenopus laevis*. Work from several groups – including those of Drs. Hoatlin, Gautier and Walter – recently led to important insights into the recruitment and function of FA proteins at the replication fork [2–4]. In particular, Dr. Walter's group is able to recapitulate replication-dependent ICL repair *in vitro* using *Xenopus* egg extracts and a site-specific DNA lesion [2]. This study revealed novel information about how cells actually remove ICLs from DNA.

Finally, Drs. Muller and Williams provide an assessment of current and future strategies for the treatment of hematopoietic failure in FA. Ninety percent of FA patients die as a consequence of bone marrow failure. These authors provide a comprehensive review of hematopoietic stem cell (HSC) defects in FA patients and therapeutic options, including allogeneic HSC transplant and gene therapy. Several factors make FA an ideal disease for treatment by gene therapy. First, in each patient it is monogenic. Second, a single organ system (hematopoietic) is life limiting. Finally, hematopoietic cells can be cultured and expanded *ex vivo*, making viral transduction with a corrective gene possible. Nevertheless, FA patients present unique challenges to gene therapy strategies. For instance, HSCs from FA patients are hypersensitive to culture conditions and FA patients are hypersensitive to the ablative therapy necessary to purge the bone marrow of potential cancer cells.

Obviously, not all important issues related to FA can be addressed in a single special edition. In future research, we anticipate seeing greater focus on the role of FA proteins as tumor suppressors in the general, non-FA population. This, and related topics, have been detailed in recent reviews or papers. For example, the mutation of FA genes, or their inactivation through promoter hypermethylation, has been linked to a variety of tumors [5–7]. Furthermore, because of the important role of FA proteins in the repair of ICLs, they may offer novel targets for cancer therapy [8,9]. Another issue warranting further research is the characterization of common spontaneous cytogenetic abnormalities in FA and how this is related to leukemogenesis [10]. Bone marrow transplantation is counter-indicated in patients with these non-random cytogenetic abnormalities due to the risk of evolving leukemic clones. It is also important to characterize such cytogenetic abnormalities since identification of genes at these breakpoints could help identify novel oncogenes or tumor suppressors.

This special edition is instead focused on how FA is diagnosed, on the function of FA proteins, on FA phenotypes, on model organisms for understanding the role of FA proteins in DNA repair, and on the treatment of hematopoietic failure in FA. It is our hope that this issue will be useful to researchers and clinicians working on FA, and to others working in the domains of genetic instability, DNA damage response signaling, DNA repair, and experimental therapeutics. It is also our hope that the papers contained within this issue will be informative for future basic research and clinical studies.

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