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Molecular Mechanisms of Fanconi Anemia

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Chapter 7

PREFACE

Fanconi anemia (FA) is a rare largely autosomal recessive genetic disorder (one complementation group being X-linked) that was first recognized almost 40 years ago[5] as a cause of juvenile leukemia. Other phenotypes include bone marrow failure leading to aplastic anemia, growth retardation, congenital malformations of renal, cardiac, skeletal and skin structures, pancytopenia and pronounced cancer predisposition.

Interestingly FA shares a number of clinical and molecular features with a variety of other syndromes, including Seckel syndrome[1] and Nijmegen breakage syndrome,[6] and further investigation of all three conditions will provide insights into the molecular mechanisms involved in each one and where they may interact.

In the recent past much effort has gone into understanding the molecular pathogenesis of FA in terms of enhanced susceptibility to DNA damaging agents. Results of these studies have yielded exciting information on a multiplicity of hitherto unknown protein-protein interactions involved in activities from control of redox state and apoptosis to repair of DNA strand breaks.[15]

Working with the leading researchers and clinicians in the field, this book has been produced to provide a comprehensive treatise on FA. This covers in detail what is known of the 12 complementation groups identified to date. These include FANCA, -B (X-linked, localized at Xp22.31),[11] -C, -D1, -D2, -E, -F, -G, -I, -J, -L and M.

There is clear variation in the clinical features exhibited by sufferers in different complementation groups, and even between those with different mutations of the same gene. This is explored in detail in Chapter 1 and expanded upon in the introduction to Chapter 5. The link between mutation and phenotype may ultimately help pick apart the complex cellular actions of the FANC proteins. At the other end of the spectrum and the book, Chapter 10 details the best treatments as currently perceived for FA. Whilst the future holds out the possibility of gene therapy or protein replacement, the treatment of choice at present is still stem cell or bone marrow transplantation from a matched sibling.[7,8]

Chapter 3 introduces the *FANC* genes identified to date and their products. It is apparent that FANCA, -B,-C,-E, -F, -G and -L form a core complex within the nucleus of cells leading to monoubiquitination of FANCD2. ATR checkpoint kinase and RPA1 are required for efficient FANCD2 monoubiquitination.[1] The nature and roles of this complex are the subjects of Chapter 7. FANCD1 and FANCJ appear to act downstream, being actively recruited along with BRCA1 and RAD51 to chromatin at specific foci for the repair of DNA damage. The precise role of FANCI has not yet been determined but it seems to act at a stage between core complex formation

and FANCD2 ubiquitination.[10] The involvement of breast cancer susceptibility and FA genes in this pathway is an area of active research. It is evident that FA-D1 patients are predisposed to breast cancer,[16] although members of the other FA complementation groups do not show a high frequency of this malignancy.[14] This would seem to indicate that the BRCA proteins may exert their breast cancer preventative function independently of FANCD2 ubiquitination (although see below). BRCA1 and BRCA2 are known to be independently involved in other activities including regulation of differentiation, and it may be that the disruption of these impacts on breast cancer development.[9]

In addition to their central role in DNA repair the FA proteins are intimately involved in a range of other cellular functions through their individual interactions with other proteins. This concept is introduced in Chapter 2 where proteins interacting with FANCA, FANCC and FANCG are introduced. Several of these interactions are with proteins involved in maintenance of redox balance. In Chapter 9, Pagano and Ahmad, long term proponents of the oxidative stress theory of FA, expand upon the ways in which interruptions in FA-redox protein associations could result in the phenotypic abnormalities observed in FA, providing an alternative model to the more widely accepted DNA repair deficiency. Abnormalities in mitochondrial function in FA further support a role of oxidative stress.[4] It is likely that it is a combination of a deficiency in redox control and ineffective DNA repair which give the complex clinical characteristics of this disorder: many of the congenital abnormalities arising due to the former, and predisposition to malignancy to the latter. It is highly probable, however, that the debate over the relative contributions of these two phenomena will continue for some time.

Mutations in FANCA, -C and -G are the most prevalent and the individual genes and their products are considered in detail in Chapters 3-5. Interestingly other proteins which FANCA, -C and -G interact with are known or suggested to be involved in chromatin remodeling (e.g., FAZF[13] and BRG1),[12] and FANCC in particular appears to have a role in protection against cytokine-induced apoptosis in haematopoietic cells. These phenomena are discussed in detail in Chapters 4 and 8 and evidence is beginning to appear to suggest that different structural domain within FANCC are responsible for its different activities. FANCG contains sequence motifs typical of a protein involved in protein-protein interactions, and it is essential for the functional interaction between FANCC and FANCA. In turn its ability to interact with FANCA requires the presence of FANCB, -C and -F, illustrating further the intricate interplay of these proteins which is explored in detail in Chapter 5.

Chapter 6 considers the natures of FANCD1 and -D2 proteins. The exciting discovery that FANCD1 is in fact the breast cancer susceptibility gene

product BRCA2, and that ubiquitinated FANCD2 interacts with BRCA1 have hugely expanded the research efforts in this area. The nuclear FA complex appears to exert its effects in a two pronged approach: by ubiquitination of FANCD2 which in turn recruits BRCA1 to the nuclear foci formed at DNA repair sites, and via interactions (possibly through FANCG association) with BRCA2 and RAD51, these proteins also being recruited to damage foci although seemingly independently of BRCA1.[17] The detail of these interactions and their roles in damage repair are under intense investigation, particularly as BRCA1 recruitment seems to be implicated in a range of DNA repair mechanisms.[15]

The final chapter (Chapter 12) is devoted to an in depth discussion of the mutations found in a geographically and genetically isolated Japanese FA population. Interestingly this group shows a preponderance of mutations in FANCA as is observed in the West, but also shows a higher frequency of FANCG involvement, confirming the central role of FANCA and the FANCA-FANCC-FANCG complex in protection against FA.

A recent Tunisian study of 41 families has revealed that 92% belong to the FANCA complementation group.[2] Others have identified Spanish Gypsies as the ethnic group with the world's highest prevalence of FA—the carrier frequency being 1/64-1/70.[3] Affected individuals are homozygous for a specific FANCA mutation (295C>T) leading to FANCA truncation. This mutation was not found in other Gypsy patients from Hungary, Germany, Slovakia and Ireland. In a Southern African population (South Africa, Swaziland, Mozambique and Malawi) a particular deletion mutation in FANCG (c.637 643delTAACCGCC) has been reported in 82% of FA patients. Birth incidence of FA in this population is greater than 1/40,000 which is above average in general populations. These studies are clearly indicative of founder mutation in the Spanish gypsy and Southern African populations.

There is no evidence that interest in FA is waning, and this book should provide both the experts and novice researchers in the field with an excellent overview of the current status of research and pointers to future research goals.

Note Added in Proof

In a recent study Meetei et al have identified a new complementation group, FANCM. The protein, FAAp250, is a part of the FA protein complex involving BRCA1 and BRCA2 and has sequence similarity to other known DNA repair proteins, including Hef in archaea, MPH1 in yeast and ERCC4 or XPF in human. FANCM is essential for monoubiquitination of FANCD2 and is hyperphosphorylated when DNA is damaged.

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