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Advances in the understanding of the Fanconi anemia tumor suppressor pathway

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Extremely high cancer incidence in Fanconi anemia (FA) patients has long suggested that the FA signaling pathway is a tumor suppressor pathway. Indeed, our recent findings, for the first time, indicate that the FA pathway plays a significant role in suppressing the development of non-FA human cancer. Also our studies on FA group D2 protein (FANCD2) have, among the first, documented the crosstalks between the FA and Rad6/Rad18 (HHR6) pathways upon DNA damage. In this review, we will discuss how our studies enhance the understanding of the FA tumor suppressor pathway.

Fanconi anemia (FA) is a rare autosomal recessive disease, presenting many developmental abnormalities and an extremely high incidence of both hematological and non-hematological malignancies.[1-5] At the cellular level, FA is characterized by chromosomal breakages and hypersensitivity to DNA crosslinking agents.[1,4,6-8] The similar sensitivity of FA cells from 16 groups (FANC-A, B, C, D1, D2, E, F, G, I, J, L, M, N, O, P, and Q[9-18]) and their characteristic clinical phenotypes suggest that FA proteins all function in a common signaling transduction pathway (the FA pathway). This pathway is activated during DNA replication or upon DNA damage, especially when triggered by DNA crosslinking agents such as MMC, diepoxybutane (DEB), and cisplatin.[19-21] FANCD2 monoubiquitinated at K561 by the FA complex E3 with FANCL as a catalytic subunit works along with other FA and non-FA proteins[14,16,22-26] to repair DNA damage and/or to exert other important cellular functions yet to be explored, as such currently understudied in our laboratory. FANCD2 thus appears to be the center of the FA pathway,[10] and monoubiquitinated/activated FANCD2 can act as a representative for the functions of activated FA signaling.

The similar sensitivity to DNA crosslinking damage revealed from FA cells and yeast cells (Rad6$^{-/-}$) prompted us to explore the potential relationship between the FA and Rad6 or HHR6 (human homologs of yeast Rad 6) pathways. As a result of our pioneering studies it is now understood that HHR6 regulates FANCD2 monoubiquitination, indicating a common link between the FA and HHR6 pathways in the maintenance of genome integrity.[27]

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Following this study, we found that hRad18, a HHR6 partner, can also regulate FANCD2 monoubiquitination,[28] accompanied by the similar finding reported from other groups.[29,30] Moreover, we also found a novel function of FANCD2 that can modulate the activity of translesion DNA synthesis, at least partly through polymerase eta (pol eta). To know how FANCD2 exactly regulates pol eta function, we systematically characterized their interaction. We found that wild-type (wt) FANCD2, but not K561R (mt) FANCD2, can interact with pol eta at regions known for the interaction with PCNA.[31,32] Importantly, we revealed that the interaction of pol eta with FANCD2 occurs earlier than that with PCNA upon DNA damage. Further studies indicate that FANCD2 monoubiquitination plays a similar anchor role as histone to bind DNA in regulating pol eta.[4] Therefore, in the early phase of DNA damage response, FANCD2 plays crucial roles in recruiting pol eta to the sites of DNA damage for repair. Taken together, our work for the first time indicates the convergence of the FA and HHR6 pathways upon DNA damage, unveiling a novel mechanism by which FANCD2 acts as a tumor suppressor.

Germline FA gene mutations have been directly associated with many cancers including breast, ovary and pancreas owing to the defects relating to FANCD1/N/C and/or /G.[10,23,25,33,34] Specifically, mutations in FANCD1 (also called BRCA2) have an 82% lifetime risk of breast cancer, and 23% risk of ovarian cancer.[25,34] These genetic studies support Dr Swift's prediction, made more than 40 years ago, that FA heterozygotes have an increased risk of cancer and provide further support to the concept that FA proteins play important roles as tumor suppressors.[35] Somatic inactivation of the FA pathway could stem from impairment of any of the FA proteins. Among these, the hypermethylated FANCF promoter, which inactivates the FA pathway, was found in 6.7% to 30% of tested tumor cell lines, including testis, lung, ovarian, and cervical cancer lines.[36-38] Reduced levels of FANCA and FANCD2 in acute myeloid leukemia (AML) and breast cancer, respectively, have also been reported, although the causes of these reduced FANCA and FANCD2 levels remain unknown.[39,40] Collectively, the overall functional heterozygosity of the FA pathway and the downstream direct or indirect effects on FA proteins may make a more significant contribution to the increased risk of cancer, particularly as many alterations appear to occur in tumor cells, than genetic heterozygosity in FA genes.

Several years ago, the involvement of the FA pathway in non-FA cancers was not well understood. We thus started to reveal the potential relationship between the FA pathway and non-FA

human cancer. We found that the non-FA Calu-6 lung cancer cells carries a defective FA pathway.[41] The examination of 6 FA proteins, essential for the activation of the FA pathway, detected substantially reduced expression of FANCL, a catalytic subunit of the ubiquitin ligase/E3-complex, indicating functional heterozygosity of the FA pathway can be attributed to the reduced FANCL expression. The continuation of studying non-FA Calu-6 cancer cells lead to a finding of a novel tumor promotion factor named "FAVL", a variant of FANCL. Subsequently, we went on a thorough investigation into how FAVL changes the FA pathway and how the altered pathway promotes development of non-FA human tumors. We found that FAVL expression was elevated in half of the human carcinoma cell lines and carcinoma tissue samples tested (nearly 100 human tumor samples tested). Expression of FAVL resulted in decreased FANCL expression in the nucleus by sequestering FANCL to the cytoplasm and enhancing its degradation. Importantly, this impairment of the FA pathway by FAVL elevation provided human cancer cells with a growth advantage, caused chromosomal instability in vitro, and promoted tumor development in a xenograft mouse model. Our studies, for the first time, indicate that FAVL impairment of the FA pathway substantially contributes to the development of non-FA human cancers and therefore add a challenging layer of complexity to the pathogenesis of human cancer.[42,43]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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