A new look at molecular biology of breast cancer

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ABSTRACT
In the past 25 years, incidence rates of breast cancer have risen about 30% in westernized countries. Mutations in BRCA1 and BRCA2 are the most prominent cause of breast cancer. However, these cancer susceptibility genes (BRCAs) only account for a few percent of women suffering breast tumor. With our understanding that BRCAs are Fanconi Anemia (FA) genes, investigations into the FA signaling network should provide a previously unrecognized key to unlock in-depth insights into both etiology and treatment of breast cancer. Here, we discuss utilization of the FA signaling as a unique genetic model system to expand our knowledge about the molecular biology of breast cancer and potential applications of the gained knowledge to enable preventive and therapeutic approaches for breast cancer patient care.

Brcas and the FA signaling network

The Fanconi Anemia (FA) pathway is composed of at least twenty-two FA gene-encoded proteins (FANC-A/B/C/D1/D2/E/F/G/I/J/L/M/N/O/P/Q/R/S/T/U/V/W) to guard against chromosomal instability. Within this pathway, FANCS, FANCD1, FANCJ and FANCN are BRCA1, BRCA2, BRIP1 and PALB2 respectively, which are breast cancer susceptibility genes (BRCAs) (also contributing to the susceptibility of other cancers). The BRCA-encoded proteins work mostly at the downstream of the FA pathway in concert with the activated/monoubiquitinated FANCD2 and its paralog FANCI to perform the signaling-transduction and/or DNA-damage repair, enforced by checkpoint mechanisms upon a variety of genotoxic stresses.1-5 Given the fact that the malfunctioned FANCD2 confers the molecular defects for more than 98% of FA cases,1 studies on FANCD2 become increasingly attractive. It sits right at the center of the FA signaling pathway, orchestrating nearly all individual players in the FA signaling pathway. FANCD2 can be an important “successor” to process upstream signaling as early as damage/stress-sensing, such as the roles played by FANCM and/or FANCW in modulating ATM/ATR effects. Many other FA proteins & FAAPs also act at the upstream of FANCD2 by performing E3 ligase activity together in the protein complexes and/or modulating E3 activity in a complex-dependent or -independent manner. FANCD2 can also be called as a critical “savior” for the completion of damage-control performed by BRCA gene-encoded products along with many other FA and non-FA proteins that appear to be in the downstream of the FA signaling. These include conducting nearly all putative phases in cell surveillance mechanisms;6-8 such as passing damage signaling and performing various types of DNA lesion repair (base or nucleotide excision repair, post-replication repair, homologous recombination, non-homologous end joining4,9). Therefore, each player involved in the FA signaling shall play roles as important as those conducted by BRCAs in the maintenance of genome stability; however, the majority of FA-signaling players are largely overlooked in regards to their characteristics implicated in breast cancer.

New insights into FA signaling network

Most cases, the onset of cancer depends on mutations in genes involved in genome “care-taking” processes.6-8 The known human genetic syndromes including FA or engineered mouse mutants have demonstrated biological significance of care-taking genes. As mentioned above, five major multi-step DNA repair mechanisms are all parts of the FA signaling network, which can act as a big care system to look after various types of errors/lesions possibly occurring on DNA. With accumulated researches on FA signaling, our understating of FA signaling is constantly extended by the new connections or interplays recognized. Noting that interplays with other cancer susceptibility genes that are involved in Bloom syndrome, xeroderma pigmentosum variant (XPV);13,14 or partnership with FAN1 and others in executing essential cellular processes. Importantly, a considerable amount of effort has been put in revealing mechanisms underlying hypersensitivity to a variety of DNA damage agents and genome instability associated with increased cancer risk. This has also disclosed a number of DNA repair and cell cycle control systems that are closely integrated with the FA signaling, as such ATM phosphorylating FANCD2 at S222,15 ATR as a kinase for FANC1 and partnership of MCM complexes with FANCD2.19 Based on our studies, the following is to show how our understanding of FA signaling was expanded.
**Insights for the upstream FA signaling**

The similar sensitivity to DNA crosslinking damage revealed from FA cells as well as rad6-null yeast cells (rad6/-) prompted us to examine the potential link between the FA and Human Homologs of yeast Rad 6 (HHR6) pathways. In this HHR6 pathway, HHR6 activates PCNA, which in turn, regulates translesion synthesis (TLS) DNA polymerases including pol η (mutated in XPV cancer susceptibility syndrome).\(^\text{25}\) Following this study, this link was further validated by a fact that hRad18 (a HHR6 partner), can also regulate FANCD2 monoubiquitination,\(^\text{26}\) accompanied by the similar finding reported from other groups.\(^\text{27}\,\text{28}\) As a consequence, it is now understood that HHR6 regulates FANCD2 monoubiquitination, which becomes a common link between the FA and HHR6 pathways in guarding genome integrity.\(^\text{10},\text{20}\) These findings indicated the convergence of the FA and HHR6 pathways upon DNA damage at the activation of FANCD2, unveiling an additional molecular mechanism underlying tumor suppression employed by the FA signaling pathway as well as the HHR6 pathway.

Bloom Syndrome (BS), similar to FA, is an autosomal recessive DNA-repair deficiency disease that exhibits chromosomal instability and a high incidence of cancer.\(^\text{25}\,\text{26}\) The BLM protein (mutated in BS) provides instructions for making a member of a protein family called RecQ helicases,\(^\text{27}\,\text{28}\) which bind to DNA and temporarily unwind the two spiral strands of the DNA molecule. This unwinding is essential for replicating DNA in preparation for cell division as well as for repairing damaged DNA.\(^\text{29},\text{30}\) Both FA and BS genetic disorders share a partial-overlap phenotype, suggesting a functional interaction between BLM and the FA pathway. Indeed, studies on sharing the common protein complexes or being a functional partner, to some extent, tested the functional interactions.\(^\text{31}\,\text{32}\,\text{33}\) Among these, a previously unknown link supported by the delayed activation of FANCD2 upon DNA damage in BLM deficient cells,\(^\text{11}\) further demonstrated the essential interplays between or among cancer susceptibility gene-encoded products in the early response to genotoxic stresses.

**Implications of FA signaling in etiology and treatment of breast cancer**

Following the functional demonstration of impaired FA signaling contributing to the development of human cancer in patients without FA,\(^\text{41}\,\text{42}\) the genetics and metabolomics studies further confirmed this unique role in promoting formation of human cancers,\(^\text{43}\,\text{44}\) including breast cancer, for the general population. BRCA2 are widely accepted to be important tumor suppressors before the term of the (canonical) FA signaling pathway, which was seemingly fine for studying their individual roles in DNA damage repair. However, when enabling the gained knowledge for the clinical use, it is apparently biased to leave behind the rest of members of the FA signaling network. Therefore, when considering how mtBRCAs impact the molecular biology of breast cancer or many other types of human cancer, other mutated members would have a similar impact on those tumors even carrying mtBRCAs. Around 50% of breast cancers was found to harbor an impaired FA pathway at the genetic level,\(^\text{40}\) which may result in similar molecular and biological effects as mtBRCAs and contribute to breast tumorigenesis. In clinic, several different tests aiming at mtBRCAs are available for helping mitigate malignant outcomes.\(^\text{2}\) Some tests look for a specific mutant form of BRCA1 or BRCA2 that was already identified as another family member; and other tests check for the known mutations. In addition, multi-gene-testing utilizes next-generation sequencing to spot for harmful mutations simultaneously in many genes that are associated with an increased risk of breast cancer, including BRCA1 and BRCA2. The positive testing results are now even used effectively in the protection of people from getting breast cancer. Such as prophylactic mastectomy and/or oophorectomy to block the...
On the other hand, mtBRCAs have also been a better prognosis for breast cancer patients treated with DNA-damage related regimens. Given such impact of mtBRCAs, we believe that many other mutated FA genes shall possess similar influences on a subject tumor, even carrying wtBRCAs. For this, we herein propose to use “impaired-FA-signaling carriers” for breast tumors (or other types of tumors) that may carry wild type or mtBRCAs. As a consequence, the subject molecular categorization can help recognize alternatives for breast cancer prevention, diagnosis and treatment. Unfortunately, studies on the application of impaired FA signaling for the clinical uses are under performed. It is not too hard for us to anticipate how it would affect breast cancer if all FA genes are included in the multigene-test for genetic consultation. In addition, our understanding of the FA signaling can also help additional specific tests, which are potentially comparable to those built upon mtBRCAs. For example, the naturally existed mtBLM has been found to compromise the timely activation of the FA signaling (FANCD2 monoubiquitination), which can be translated into a biomarker for an increased cancer susceptibility and, thus, leading to a possible use for the genetic consultation, as well as the prognosis of breast cancer patients when they are subjected to therapeutic plans aiming at DNA damage.

**Conclusions**

Different from the recent reviews on FA signaling, this review emphasizes an aspect of breast cancer research that is under-investigated. There is a lot that remains to be addressed as illustrated (Figure 1). However, this is not an obstacle that can block us to have a new look at molecular biology of human cancer, particularly breast cancer. As known, BRCA1/2 studies have been capturing the frontier of cancer research since decades ago, also made the FA signaling pathway be very attractive for a decade. However, the FA signaling remains to be “capped” by “the rare population” in the field of cancer research. As a result, in-depth understanding of the etiology and treatment of human cancer, particularly, breast cancer, appears to mostly depend on the understanding of BRCAs, not on the team-work that was performed by the whole FA signaling. Here we hope the future translational

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**Figure 1.** The outline of the FA signaling network. At least 22 FA proteins can work in a common signaling network to protect cells from going awry for diseases, including cancer. The updated portion in gray lines via our published work. Overall, if the functions played by this signaling network are compromised, tumorigenicity or the sensitivity to DNA-damaging therapies will be promoted. This is not limited to the mtBRCA carrying tumors (in red).
studies on each player in the FA signaling network will be considered properly, thereby enabling the relevant knowledge to be more helpful for the prevention, diagnosis, or treatment of breast cancer.

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