Commentary

Keap1/Nrf2 impairing revised: are we missing the single nucleotide polymorphisms?

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Oxidative stress represents one of the main causes of DNA damages in cancer development and it is mainly modulated by the Kelch-like ECH-associated protein 1 (Keap1)/Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway that leads the mechanism of cellular adaptation to oxidative and xenobiotic stresses. The Keap1 protein is the main negative regulator of the Nrf2 transcription factor level (1). In physiological conditions, Keap1 protein binds to cytoplasmic Nrf2 and modulates its protein levels by inducing ubiquitination and cytoplasmic proteasomal degradation. Under cellular stress conditions, the Keap1 is subjected to a conformational change that reduces its ability to interact with Nrf2. *De novo* synthetized Nrf2 translocates then into the nucleus and binds the antioxidant response elements (ARE) to the gene promoters of downstream cytoprotective factors, such as Phase II detoxifying enzymes, drug transporters, anti-apoptotic proteins and proteasomes (2).

In the recent years, the key function that Keap1 exerts in the negative modulation of the Nrf2-mediated intracellular redox-balancing, but also in Bcl2-mediated apoptosis, inflammation, p62-mediated autophagy, lipid and glucose metabolism (3) have been corroborate by a number of experimental evidences.

In cancer cells has been observed an aberrant function of the Keap1 protein, with a consequent increasing number of unbound Nrf2, which accumulates into the nucleus and mainly enhances the tumorigenesis process and tumor resistance to adjuvant treatments (4).

Therefore much more attention has been paid lately to the role of Keap1 in the cancer pathology. Genetic and epigenetic mechanisms of deregulation of the Keap1/Nrf2 pathway have been described in solid tumors of different histogenesis, and have been evaluated in terms of their impact both on prognosis and response to chemo- and radio-therapy (5,6). Somatic mutations of the *KEAP1* and *NFE2L2* genes and/or aberrant *KEAP1* promoter hypermethylation were found to predominate in lung cancer, and, although less frequently, in head and neck cancer, biliary tracts cancer, gliomas and breast cancer (6-11). However, the molecular basis of deregulation of the Keap1/Nrf2 axis has not been yet fully elucidated, in spite of recent findings in other solid tumors.

Numerous genome wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with the increased risk of tumor development. Since most of the susceptibility SNPs are located in noncoding regions and they do not disrupt coding sequences, little is known about their functional mechanisms. In many cases, SNPs reside in cell type-specific regulatory elements that mediate the binding of critical transcription factors (TFs), which in turn result in changes in target gene expression (12) Recently, tumor susceptibility SNPs were also associated to miRNA/lncRNA

binding regions (13) or sequences of protein-protein interactions (14).

In this subject, the recent work by Hartikainen and coworkers, published in Clinical Cancer Research, explored more in depth the association of five *KEAP1* SNPs with clinical breast cancer and reported for the first time the potential correlation among SNPs, breast cancer risk and prognosis in terms of progression free survival (PFS) and overall survival (OS) (15).

This case-control study is referred to a large Caucasian cohort of about 1,000 subjects from two different population-based sample sets from Finland: the Kuopio Breast Cancer Project (KBCP) and the Northern Finnish Oulu Brest Cancer Study (OBCS). As a result, all the five investigated SNPs appeared to be in allelic linkage disequilibrium (LD), thus suggests an existing haplotypes block into the *KEAP1* gene locus that might correlate with specific clinical features. In particular, the two SNPs rs9676881 and rs1048290 resulted to be significantly associated with a shorter PFS survival in both invasive breast cancer and ER-positive tamoxifen-treated invasive breast cancer patients. The physical location of these two SNPs into the *KEAP1* gene might explain the observed correlation with a higher Keap1 protein expression level and a high cytoplasmic localization of Nrf2 in breast tissues. Indeed, the SNPrs1048290 is located into the genomic region encoding the DGR domain (responsible for the interaction of Keap1 protein with cytoplasmic Nrf2) so it may effects the maintenance of physiological levels of Nrf2 protein. More interestingly, this SNP has been found in LD with the SNP rs9676881, which is located in a putative enhancer region, few bases downstream of the 3'-untraslated region (3'-UTR) of the *KEAP1* gene, and 410 bp from the miR-200a binding site. By consequence, the Authors suppose that a LD may exist also with additional unexplored silent variations that affect this specific miRNA binding site. In fact, miR-200a family, including miR-200a, miR-200c, miR-141, has been found downregulated in breast cancer, as involved in the regulation of epithelial phenotype and, as such, in epithelial to mesenchymal transition processes (16). Loss of miR-200a in MDA-MB-231 and Hs578T breast cancer cell lines has been just proved to enhance the Keap1 expression and to reduce consequently the Nrf2 cytoplasmic level in mammary epithelium (17). The main alteration of Keap1/Nrf2 axis, reported so far in breast cancer, is the promoter hypermethylation (9). Therefore, one of the most intriguing consideration, arising from the work of Hartikainen and colleagues about the SNPs location, is that miRNA could represent a possible additional epigenetic way to deregulate the Keap1/Nrf2 pathway in breast cancer.

This study gives the first hint of the prognostic role that apparently silent variations in the *KEAP1* gene may acquire in breast cancer without inducing any evident and detectable protein structural or conformational variation. Since these variations are common polymorphisms in the population it is very intriguing to screen these SNPs and to study their potential role also in other solid tumors where the Keap1/Nrf2 pathway is already found impaired.

In light of this, further additional preclinical and clinical studies are recommended in order to confirm the present findings as well as to evaluate the clinical utility of SNPs and the putative link with miRNAs in different tumors and populations from multiple geographical locations.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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