Mutational assessment of preinvasive lesions of breast ductal carcinoma

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Background and objective

Ductal carcinoma is represented by ductal carcinoma *in situ* (pure DCIS) and invasive breast carcinoma of no special type (IBC-NST), the last can be detected concurrent with an *in situ* component (*in situ* of IBC). DCIS is a preinvasive lesion which accounts for approximately 25% of all breast cancers, it is a heterogeneous tumor and most of them are indolent, but some can progress and relapse. It has encouraged the seek for biomarkers able to stratify patients according to the risk of progression or relapse, and consequently to identify patients at a lower risk who could avoid an overtreatment. The aim of this study was to perform a mutational characterization of preinvasive lesions of breast ductal carcinoma, to identify potential biomarkers of DCIS progression or relapse.

Methodology

The cohort was composed by patients diagnosed as pure DCIS at anatomopathological report of biopsy and presenting different outcomes afterwards (**Figure 1**). Group 1: DCIS at anatomopathological report of both biopsy and surgical specimen and with no progression or relapse after ≥ 3-year follow-up. Group 2 was divided into 2 subgroups. Group 2.1: DCIS at anatomopathological report of biopsy and IBC-NST with *in situ* component at anatomopathological report of surgical specimen. Group 2.2: DCIS at anatomopathological report of both biopsy and surgical specimen and with progression or relapse during the follow-up. Somatic mutational profiling was performed by a 409-gene panel (CCP) and by a 50-gene hotspot regions panel (CHP2). NGS sequencing was performed on Ion Torrent platform. Potentially somatic variants were called by TVC, and annotation was performed by consulting different databases. Our criteria were: sequencing: ≥ 50% reads on target; mean coverage ≥ 500x; variant calling: coverage ≥ 100x, VAF tumor ≥ 5%, VAF leucocytes < 0,3%, MAF < 0,5%, seek for variants in oncogenes/tumor suppressor genes (TSG); variants selection: splice site, missense and LoF.

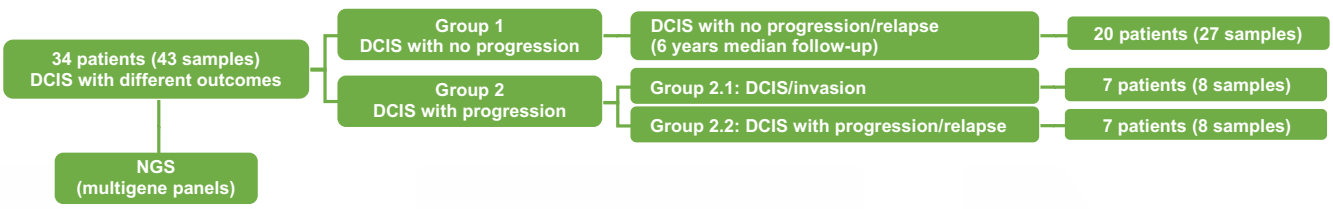

Figure 1: Experimental design for the assessment of the mutational profile of preinvasive lesions of breast ductal carcinoma.

Results

A total of 34 patients (43 samples) were selected: 20 and 14 from groups 1 and 2, respectively. All 43 samples were submitted to DNA extraction, of which 36 samples were qualified for NGS with multigene panels. From 34 patients, 23 patients (29 samples) presented sequencing data qualified for single nucleotide variant (SNV) analysis. From 23 patients, a total of 16 patients was found carrying at least one potentially somatic variant, 53% (8/15) of the patients from group 1 and 100% (8/8) of the patients from group 2. From 106 potentially somatic variants found in these 16 patients, 45 variants were found in 40 oncogenes or TSG. Oncogenic and probably oncogenic variants detected in these genes were found in a higher proportion of patients from group 2 (DCIS with progression), in which 33% (5/15) and 75% (6/8) of patients were from groups 1 and 2, respectively (**Figure 2**). Frequently mutated genes in breast cancer, such as *TP53*, *PIK3CA* and *GATA3*, were also the most frequently mutated genes in our cohort (**Table 1**). Potentially somatic variants in *TP53* and *PIK3CA* genes were also found in a higher proportion of patients from group 2, in which *TP53* was mutated in 6,6% (1/15) and 25% (2/8) of the patients from groups 1 and 2, respectively; and *PIK3CA* was mutated in 0% and 25% (2/8) of the patients from groups 1 and 2, respectively (**Figure 3**).

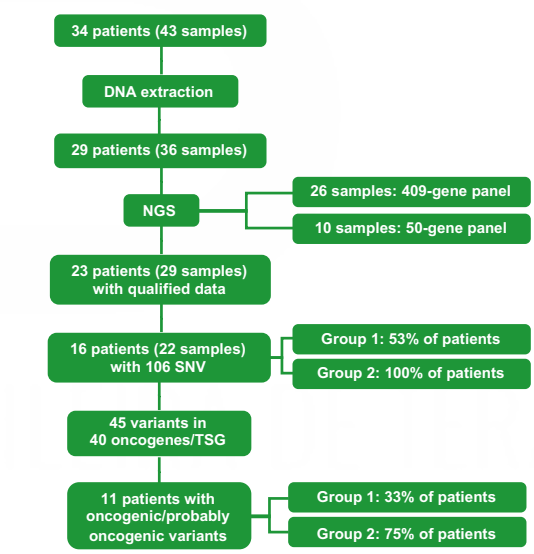

Figure 2: Workflow of the identification of the potentially somatic variants in DCIS.

Most frequently mutated genes	Frequency of mutation
TP53	13% (3/23)
PIK3CA, GATA3, *CIC, EGFR, TET1*	8.7% (2/23)
AKT1, *ALK, ATM, BRAF, DDR2, ERCC3, ERCC5, ETV4, HIF1A, IKBKB, JAK3, KMT2A,* ***KMT2C***, *KMT2D, LRP1B,* ***MAP2K4***, *MRE11, MUTYH, NOTCH4, NSD1, NSD2, PARP1, PER1, PIK3CB, PLCG1, PML, PPP2R1A, PRDM1, RAD50,* ***RB1***, *ROS1,* ***RUNX1***, *SETD2, SMARCA4*	4.3% (1/23)

Table 1: Genes most frequently mutated in our cohort of DCIS. Genes highlighted in black are those most frequently mutated in breast cancer.

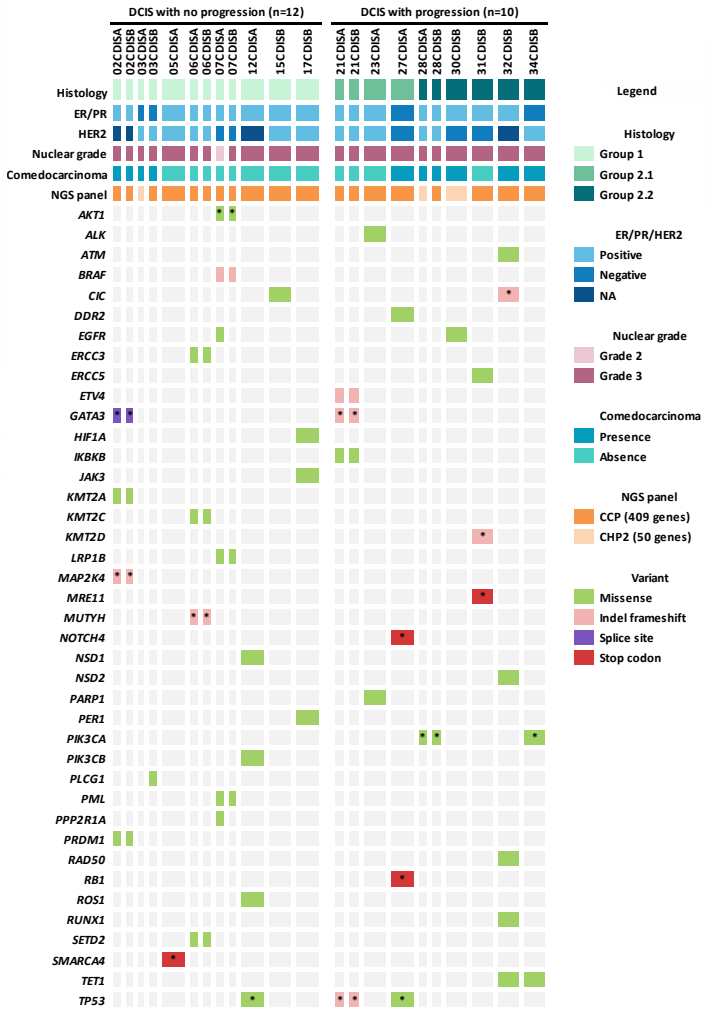

Figure 3: Mutational profile of DCIS. Suffixes A and B in the sample name represent biopsy and surgical specimens, respectively. (*) symbol represent oncogenic/probably oncogenic variants.

Conclusion

DCIS lesions of patients from group 2 (DCIS with progression) showed a higher proportion of oncogenic and probably oncogenic variants in oncogenes/TSG frequently mutated in breast cancer. However, the evaluation of a large cohort of DCIS patients who manifested different outcomes is needed to identify potential biomarkers of DCIS progression or relapse.