MYBL1 rearrangements and MYB amplification in breast Adenoid cystic carcinoma (AdCC) lacking MYB-NFIB fusion gene

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Adenoid cystic carcinoma (AdCC) of breast

- Composed of dual population of luminal myoepithelial/basal cells
- Commonly arise in salivary gland, lung, lacrimal gland and breast etc.
- Rare type of breast malignancies accounts for up to 1%
- Invariably displaying a triple negative phenotype
- Differ from conventional TNBCs
  - exhibiting indolent tumor biology and clinical course
  - lacking recurrent TP53 and PIK3CA mutations, harboring low levels of genetic instability and mutational burden

Kim et al. unpublished
MYB rearrangement, known driver of Adenoid cystic carcinoma

- Molecular hallmark t(6;9)(q22-23;p23-24) translocation creating a MYB-NFIB fusion gene, well known oncogenic driver of AdCCs
- MYB rearrangement
  - Found in ~50% of salivary gland and in 38-100% of breast AdCCs
- MYB gene
- Encodes c-MYB transcription factor, regulating expression of multiple target genes

Marchio et al. J Clin Pathol 2010
Persson et al. PNAS 2009
Oncogenic mechanism of *MYB-NFIB* fusion gene

- *MYB* family (*MYB, MYBL1, MYBL2*)
- Encodes c-MYB transcription factor, regulating expression of multiple target genes
- **Overexpression of *MYB* gene**
  - Loss of negative regulatory domain by fusion event
  - Relocation of super-enhancers originally located in *NFIB* closer to *MYB* promoter
- Recently, alternative genetic mechanisms, such as *MYBL1* rearrangements, have been reported in *MYB-NFIB*-negative salivary gland AdCCs

Aim

• To identify alternative oncogenic drivers of this rare special type of TNBC
eg. MYBL1 rearrangement recently reported in salivary gland AdCC

• Illustrate a comprehensive genomic and transcriptomic repertoire of *MYB-NFIB*
negative breast AdCCs
Methods

- Four cases of AdCCs confirmed negative of MYB-NFIB fusion gene by FISH
  - RNA-sequencing was performed to identify MYB rearrangement (N=3)
  - Whole genome sequencing (WGS) for other rearrangement (N=2)
  - Massively parallel sequencing (MSK-IMPACT) d/t tissue availability (N=1)
- Break-apart FISH for MYB/MYBL1 gene to confirm rearrangements
- Quantitative RT-PCR and RNA-sequencing for gene expression
- Expression of downstream pathways evaluated using gene set enrichment analyses (GSEA)

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Case 1; MYBL1 rearrangement (1)

- *MYB-NFIB* fusion gene negative by FISH, c-MYB positive by IHC
- *MYBL1-NFIB* fusion gene identified by RNA-sequencing
- *MYBL1* break-apart FISH confirmed rearrangement

Negative regulatory domain (NRD) preserved
Case 2; **MYBL1** rearrangement (2)

- *MYB-NFIB* fusion gene negative by FISH
- *MYBL1-NFIB* fusion gene identified by RNA-sequencing and whole genome sequencing
- *MYBL1* break-apart FISH confirmed rearrangement

Loss of negative regulatory domain (NRD)
Case 3; *MYB* amplification

- *MYB* amplification (*n=1*) found by targeted massively parallel sequencing (*MSK-IMPACT, *)
- Amplification of 6q23.3 encompassing *MYB* gene
- Overexpression of c-MYB by MYB-FISH and Immunohistochemistry

*MSK-IMPACT
Memorial Sloan Kettering  Integrated Mutation Profiling of Actionable Cancer Targets
Deep sequencing of all exons and selected introns of 410 cancer genes
Gene expression by RNA-sequencing and qRT-PCR

- **MYB/MYBL1** gene expression (Both 5’ and 3’ segment of each gene)
  - Highly expressed MYBL1, in cases with MYBL1 rearrangement
  - Highly expressed MYB, in cases with MYB rearrangement or MYB amplification
  - Only the 5’ part of the gene, segment after fusion junction not overexpressed
Downstream effect of MYBL1 rearrangement or MYBL2 intronic mutation

- Pathway analysis using ssGSEA (single sample gene set enrichment analysis)

- Compared to the reference case, MYB-NFIB positive AdCC, cases with MYBL1 rearrangement, MYBL2 intronic mutation displayed resemblance of downstream pathway activation
Conclusion

• Our data support the contention that *MYB/MYBL1* activation likely constitutes the common mechanism driving breast AdCCs, to which various underlying genomic alterations may converge.

• Further investigations of larger cohorts of AdCCs lacking the *MYB-NFIB* and *MYBL1* fusion genes are warranted to elucidate drivers other than *MYBL1* rearrangements and *MYB* amplification.
Acknowledgements

• Jorge S Reis-Filho
• Britta Weigelt
• Felipe C Geyer
• Ashwini Rhagavendra
• Kathleen A Burke
• Raymond Lim
• Pier Selenica
• Anqi Li
• Fresia Pareja
• Rodrigo Gularte-Merida
• Ashwini Raghavendra
• Rui Bi
• Thais de Oliveira
• Charlotte K Ng
• Luciano Martelotto
• Pedro Blecua
• Simon Powell
Thank you very much