GENOMICS AS A TOOL FOR DISCOVERING GENES CAUSALLY IMPLICATED IN CANCER DEVELOPMENT AND PROGRESSION

DIRCE MARIA CARRARO, PhD
AC CAMARGO CANCER CENTER
Cancer

All cancers are caused by somatic mutations

Exogenous or endogenous mutagen exposures

Germ line mutations
- BRCA1/2 – breast and ovarian cancer
- Mismatch repair genes (MSH2, MLH1 and MSH6) – colorectal cancer
- CDKN2a – melanoma

Body cells

Somatic mutations
Tumor evolution by Somatic mutation accumulation

Driver mutation: directly or indirectly confers a selective growth advantage to the cell in which it occurs.

Driver genes: three cellular processes: cell fate, cell survival, and genome maintenance

Vogelstein et al. Science, 2013

Genomics analysis
(Next Generation Sequencing)

Highly frequent variants
Driver mutations
Background

- Somatic mutation is consequence of infidelity of the DNA replication machinery
- Mutations that lead to a defective protein involved in crucial cellular functions may trigger cancer
- Biological Process that are operative in tumor can be (at least partially) revealed by knowing its somatic mutational landscape
- Advances in genomic tools
  - To assess the causally implicated genes in cancer development and progression
  - To disclosure biomarkers for diagnostic, prognostic and target therapy
Breast Cancer

- Progression of Ductal Carcinoma *in situ*
- *BRCA1* mutation and Triple Negative Breast Tumor
- Hereditary Breast Cancer

Wilms Tumor – pediatric nephroblastoma

- Factors involved in development of WT
  - Gene Expression - under the perspective of kidney development
  - Mutation repertoire of WT
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Breast Cancer

Brazil: 2014 - 57,120 new cases
~ 6,000 hereditary breast cancer

http://www2.inca.gov.br/

Hereditary Breast and Ovarian Cancer (HBOC) - an autosomal dominant disease

- **BRCA1**
  - Part of a complex that repairs double-strand breaks in DNA

- **BRCA2**
  - Genome stability

- Pathogenic mutations – 25% in women

- *BRCA1* is the most prevalent mutated genes in Brazilian patients with clinical criteria for Hereditary Breast Cancer
  - Carraro et al., 2013; Silva et al., 2014
Triple Negative Breast Cancer (TNBC) and BRCA1 mutation

Negative for progesterone and estrogen receptors expression and for HER2 expression/amplification

- 60% to 80% of \( BRCA1 \) mutation carriers develop a TNBC  
  Atchley et al, 2008

- 72% of early breast cancer patients with \( BRCA1 \) germline mutations developed TNBC  
  Carraro et al, 2013

- 8.5% - 30% of TNBC have \( BRCA1 \) mutation (regardless of family history of cancer)  

- 50% of \( BRCA1 \) mutation - early onset TNBC (< 35 yo) (small cohort)  
  Carraro et al, 2013

- To determine the prevalence of \( BRCA1 \) mutation in TNBC
Loss of function mutation in *BRCA1* gene

- Screening in 131 TNBC – one single institution study – status germline or somatic by checking in blood or normal tissue
  - Point Mutation (Ion PGM Torrent and Ampliseq *BRCA* panel)

131 TNBCs
- **11.45% (15/131) were *BRCA1*-mutated tumors**
  - **9.23% (12/130) - germline *BRCA1* point mutations**
    - 85.7% (12/14) of the point mutation observed in TNBC carriers were germline events
  - **1.53 % (2/131) – somatic *BRCA1* point mutations**

Brianese R et al., not published
Age at diagnostic and Mutation Rate
somatic + germ line mutations

- Women diagnosed with TNBC before 50 yo are under risk to harbor germ line mutation in *BRCA1* gene

Brianese R et al., not published
TNBC

- 15-20% of breast tumors
- Very aggressive tumor
- Poor prognosis and overall survival
- Higher probability of relapse
- No targeted or hormone therapies available
- Very heterogeneous group

• To investigate the molecular mechanisms that outline the two groups - BRCA1-mutated TNBC and BRCA1-wild type of TNBC

Metzger-Filho et al, 2012
Bauer et al, 2007
## Genetic landscape of TNBC

### Genomic Alterations by Whole Exome Sequencing

Blood – Normal breast tissue and tumor breast tissue

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Ferreira EN et al., not published
Pattern of somatic mutation in normal and TN tumor tissues
(coding sequence – Protein affecting)

- High-frequency mutation: ≥ 30% - Drivers mutations
- Low-frequency mutation: < 30% - Passengers mutations

- **BRCA1-mut TNBC has proportionally lower number of driver mutations**
  - Distinct process of tumor evolution

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<th>BRCA1-mutated Tumor</th>
<th>BRCA1-WT Tumor</th>
<th>BRCA1-mutated Normal</th>
<th>BRCA1-WT Normal</th>
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| p-value       | p<0.0001             | p=0.22         |

Ferreira EN et al., not published
Whole Genome DNA Methylation Analysis

- 28 TNBC - 13 BRCA1 mut and 18 BRCA1 WT samples
- high-density methylation chip – Illumina 454K (CpG methylated in the intervals -1,500 to +500 to the first ATG)

Hypermethylated in BRCA1-WT TNBC

Hypermethylated in BRCA1-mutated TNBC

Ramalho R et al., not published
Summary

- Women diagnosed with TNBC are under risk to harbor pathogenic germ line mutation in BRCA1

- BRCA1 is a Driver gene for triple negative.
  - Considering as driver gene
    - a gene that contains driver gene mutations (Mut-Driver gene) – (BRCA1 mut TNBC)
    - (under analysis) a gene expressed aberrantly in a fashion that confers a selective growth advantage (Epi-Driver gene) – (BRCA1-wt TNBC).
Breast Cancer

- Progression of Ductal Carcinoma *in situ*
- *BRCA1* mutation and Triple Negative Breast Tumor
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Wilms Tumor – pediatric nephroblastoma

- Factors involved in development of WT
- Gene Expression - under the perspective of kidney development
- Mutation repertoire of WT
Wilms Tumor

• Embryonal kidney neoplasia
• TW affects children 2-5 years of age
• 10% associated to germ line mutations
• 15% relapse
WT differs from renal adult cancer

- number of somatic mutation found in Wilms tumor is much smaller than in adult solid tumors
  - the tumor evolution process is different

WT arises from metanephric blastemal cells – unable to complete the mesenchymal–epithelial transition (MET)

Gene expression study under the perspective of kidney development:
- Genes with involved in the interruption of kidney differentiation
- Genes belonged to Wnt signaling pathway - potential driver genes for WT
WT mutation by exome-sequencing

- Few genes had been identified as causally related to WT development
  - *WT1, CTNNB1, and WTX*
  - *TP53, DIS3L2, FBXW7, MYCN, and DICER1* (occasionally reported)
    - together account for approximately 30% of WTs
  
*Whole-exome sequencing*

- Somatic mutations: present only in tumor sample

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Somatic point mutation in Drosha Gene – E1147K

- DROSHA encodes a nuclear RNase III protein that plays a central role in the miRNA biogenesis pathway.
- Drosha possesses two RNase III domains, named RIIIa and RIIIb, which form an intramolecular dimer that cleaves the 3 and 5 strands of the stem, respectively.
- Drosha E1147K located at a residue of the RIIIb domain - the affected amino acid is one of the four acidic residues that form a metal-binding (Mg2+) – essential for RNase III catalytic activity.

- E1147K is a recurrent event in WT - 20/221 WT samples (139 WT from AC Cancer Center and 82 from COG)
- Entire domains RIIIa and RIIIb – three additional mutations (two in RIIIb and one in RIIIa domain). All mutations are located in residues highly conserved throughout all eukaryotes
- E1147K represents 87% of the total DROSHA mutations

Drosha mutation characterization

- All E1147K and D1151G mutations were heterozygous
- E993K alteration was a homozygous alteration (the presence of two alleles was defined by aCGH and duplex qPCR) – potential germ line variant!

Targeted sequencing of miRNA-processing genes

(DROSHA, DGCR8, DICER1, RAN, XPO5, TARBP2, AGO1, AGO2, GEMIN4 and DDX20)

- 66 frozen WT samples (15 from ACC and 51 from COG)

Point pathogenic mutations:

- 33% (22/66) - DROSHA, DGCR8, DICER1, XPO5 and TARBP2 – MicroRNA biogenesis
- 23% (16/66) - WT1, CTNNB1, WTX, TP53, DIS3L2 and FBXW7 - genes previously described to be mutated in WT

Substantial frequency of mutations in genes involved in miRNA biogenesis:
- impaired miRNA maturation might play a pivotal role in WT.
Influence of E1447K Drosha in miRNAs expression

WT samples

Comparison of mature miRNA expression profile - TaqMan Array platform
06 E1147A DROSHA-mutated WT X non-mutated WT (in the genes of miRNA biogenesis)

- strong relationship between the miRNA profile and the presence of the E1147K mutation
- E1147K mutation leads to a predominant downregulation of a subset of mature miRNAs (green).

Comparison of mature miRNA expression profile - TaqMan Array platform
06 E1147A DROSHA-mutated WT X non-mutated WT (miRNA biogenesis)
ARTICLE
Received 21 Nov 2013 | Accepted 6 May 2014 | Published 9 Jun 2014
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OPEN

Recurrent somatic mutation in DROSHA induces microRNA profile changes in Wilms tumour

Giovana T. Torrezan¹*, Elisa N. Ferreira¹*, Adriana M. Nakahata¹, Bruna D.F. Barros¹, Mayra T.M. Castro¹, Bruna R. Correa², Ana C.V. Krepischi¹, Eloisa H.R. Olivieri¹, Isabela W. Cunha³, Uri Tabori⁴, Paul E. Grundy⁵,

E1147K was also identified by Torrezan et al.¹⁵ as a mutational hotspot¹⁵. *In vitro* pri-miRNA processing assays revealed that the Torrezan et al.¹⁵ also identified non-synonymous missense mutations in DGCR8 and TARBP2, as well as XPO5 and DICER1; how to create a null allele (Fig. 1b–d). The frequency of mutations in miRNA pathway genes and specifically in DROSHA were overall similar to that reported by Torrezan et al.¹⁵ (33% and 12%, Supplementary Fig. 5a). In contrast, DROSHA⁺/E1147K cells showed statistically significantly impaired expression of a large majority of detectable miRNAs (85 out of 139; Fig. 4d and

Dinesh Rakheja¹,²,³,⁴, Kenneth S. Chen²,⁵,⁶,³,⁴, Yangjian Liu⁵,³,⁴, Abhay A. Shukla²,⁵, Vanessa Schmid⁷, Tsung-Cheng Chang⁵, Shama Khokhar³, Jonathan E. Wickiser²,⁶, Nitin J. Karandikar¹, James S. Malter¹, Joshua T. Mendell²,⁵,⁸ & James F. Amatruda²,⁴,⁵,⁷,⁸.
Summary

- Drosha E1147K is a driver mutation in WT
- Mutation in miRNA processing genes play a role in WT development
- Interference in gene expression regulation plays a role in WT onset
Perspective: Liquid biopsy for monitoring treatment of Wilms Tumor and TNBC patients

Wilms tumor
- American - Children Oncology Group (COG) (surgery as first treatment)
- Société Internationale d’Oncologie Pédiatrique (SIOP) – Chemotherapy as first treatment - Brazil

Survival rate is 80% - despite a generally good prognosis, is associated with relapse in up to 15% of cases

- Urine collected (Before chemotherapy and surgery)
- DNA extraction (from urine collected in several steps during treatment)
- NGS Target sequencing (Gene Panel – miRNA-processing pathway - 10 genes 6 previously described as mutated in WT)
- ctDNA – circulating tumor DNA in urine and plasm
- Somatic mutation for monitoring therapy response

Target sequencing panel of genes frequently mutated in WT

65% of WT Identification of somatic mutations
Triple negative breast cancer

- 80% are treated with neo-adjuvant chemotherapy
- Very heterogeneous disease regarding chemotherapy response - Subgroup of women whose TN tumors are extremely sensitive to chemotherapy, while for most of the benefit of them is uncertain
- To assess the dynamic changes in somatic mutation repertoire that occur during chemotherapy treatment by ctDNA in plasma using target sequencing (panel of genes frequently mutated in TNBC – including list of genes provided by us and from the literature)

- A comprehensive analysis of the mechanisms of sensitivity and resistance to treatment
Funding

- Gene expression analysis of different stages of kidney and liver development and its implications in embryonic tumors.  
  FAPESP: 2006/00054-0

- Signaling pathways genes involved in the recapitulation of nephrogenesis in Wilms Tumor: definition of the mutational spectrum and characterization of the regulatory and functional aspects.  
  FAPESP: 2010/00223-1

- MOLECULAR ASPECTS INVOLVED IN THE DEVELOPMENT AND PROGRESSION OF BREAST DUCTAL CARCINOMA: Investigation of Carcinoma in situ Progression and the Role of BRCA1 Mutation in the Tumor Triple Negative. FAPESP: 2013/23277-8

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- Cecilia Costa, MD, PhD - Department of Pediatric Oncology
- Isabela Werneck Cunha, MD, PhD - Department of Pathology
- Victor Pianna Andrade, MD, PhD - Department of Pathology
- Renan Valieris – Bioinformatics/CIPE
- Eloisa Olivieri, MsC – Biobank

Biobank/CIPE – AC Camargo

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