Biomarkers in Orbital Tumors

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Biomarkers
• NIH Biomarkers definitions working group ‘98
  • Characteristic objectively measured/evaluated
  • Indicator of normal biological or pathogenic process or
  • Indicator of pharmacological response to therapeutic intervention

Biomarkers in Orbital Tumors
• Soft tissue tumors
  • Rhabdomyosarcoma
  • Solitary fibrous tumor/hemangiopericytoma
  • Lipomatous tumors
  • Ewing sarcoma/PNET
• Lacrimal gland tumors
  • Pleomorphic adenoma
  • Adenoid cystic carcinoma
  • Other less common tumors

• Indicates a change
• Genetic, epigenetic or proteomics
• Tumor microenvironment
• Correlation with
  • Disease progression
  • Potential response to certain therapy
• Support certain diagnosis
Rhabdomyosarcoma

Orbital Rhabdomyosarcoma

- Most common orbital malignancy in children
  - 10% of all rhabdomyosarcomas
- Embryonal and alveolar subtypes
- Differential diagnosis with other small round cell and spindle tumors
- Subtype – implications for treatment and prognosis


8 year-old boy presented to the ED for evaluation of progressive proptosis following an episode of left maxillary sinusitis treated with antibiotics. Denied pain and diplopia.

Orbital Rhabdomyosarcoma

- MYOD1 immunostain
- Desmin immunostain
Rhabdomyosarcoma – Alveolar subtype

- Recurrent translocations
  - t(2;13)(q35;q14) - most cases
  - t(1;13)(p36;q14) - smaller subset cases
- Juxtaposition PAX3 (chr 2) or PAX7 (chr 1) with FOXO1 (chr 13)
- PAX3- and PAX7- fusion proteins - oncogenic transcriptional activators

From The Atlas of Genetics and Cytogenetics in Oncology and Hematology – Alveolar Rhabdomyosarcoma by F. G. Barr

FOXO1: FISH

- PAX3-FOXO1 fusion positive (145 bp)
- PAX7-FOXO1 fusion positive (133 bp)

Courtesy of Dr. Rondell Graham

Rhabdomyosarcoma – Alveolar subtype
Rhabdomyosarcoma – Alveolar subtype
• Fusion-positive ARMS have frequent genomic amplification
  • PAX7-FOXO1 amplification - most common
  • MYCN oncogene amplification on 2p24
  • Amplification of a 12q13-14 region - includes CDK4 gene
  • Not unique of alveolar rhabdomyosarcoma


Rhabdomyosarcoma – Alveolar subtype
• IRS-IV study – ARMS prognosis
  • Patients with localized disease - PAX3-FOXO1 and PAX7-FOXO1-positive ARMS, had comparable outcomes
  • Patients presenting with metastatic disease - PAX3-FOXO1-positive tumors had a significantly poorer outcome than those with PAX7-FOXO1-positive tumors (4-year overall survival rate of 8% compared to 75%, p=0.0015)


Rhabdomyosarcoma – Embryonal Subtype
• No structural chromosomal rearrangements
• Cytogenetics - frequent chromosomal gains
  • Chromosomes 2, 8, 11, 12, 13 and 20
• Frequent loss of one or more contiguous chromosomal 11 loci in tumor cells
  • Most frequent 11p15.5
  • Inactivation of tumor suppressor genes

Barr FG. Atlas Genet Cytogenet Oncol Haematol 2009

Rhabdomyosarcoma – Embryonal Subtype
• Important oncogenic pathways
  • Deregulation of the RB1 and p53 pathways
  • Activation of the RAS pathway
  • Activation of the hedgehog signaling pathway
  • PIK3CA and CTNNB1 (Beta-catenin) mutations
• Potential targets for future therapy

Paulson V et al. Genes Chrom Cancer 2011;50:397

Solitary Fibrous Tumor
• Composed of spindle to oval cells
  • Patternless arrangement, rich in collagen and positive for CD34 immunostain (non-specific)
• SFT- giant cell angiofibroma - hemangiopericytoma
• Neoplasms of variable clinical behavior
  • Benign behavior – majority
  • Recurrence and metastases – 5-10%
SFT – Differential Diagnosis
- Benign spindle cell tumors
  - Schwannoma, soft tissue perineuriona, spindle cell lipoma, cellular angiofibroma
- Spindle cell lesions with aggressive behavior
  - Fibromatosis
- Spindle cell sarcomas
  - Malignant peripheral nerve sheath tumor, dermatofibrosarcoma protuberans, synovial sarcoma

Orbital Solitary Fibrous Tumor (SFT)
- Review cases seen at AFIP (1970-2009)
  - 41 collagen-rich fibroblastic orbital tumors (hemangiopericytoma, fibrous histiocytoma, giant cell angiofibroma)
    - 23 males/17 females – mean age 40.7yrs
    - Orbital mass and/or proptosis, with/without pain
  - All tumors were CD34+, with variable p53 (85%), CD99 (67%), Bcl-2 (47%) and Ki67 (<1-54 %)

Solitary Fibrous Tumor (SFT)
- *NAB2-STAT6* gene fusion – majority of cases
- *NAB2* and *STAT6* genes
  - Located closely in chromosome 12
  - Normally transcribed in opposite directions
  - Fusion product results from inversion at 12q13
  - Fusion protein – interleukin-4 induced (STAT6)

Solitary Fibrous Tumor (SFT)
- STAT6 – transcriptional activator
- Induce expression of EGR-target genes
- Result in increased proliferation
- STAT6 - nuclear expression
  - Detected by immunostain
  - Specific of solitary fibrous tumor
  - Rarely expressed in other neoplasms
Solitary Fibrous Tumor (SFT) – nuclear STAT6

| Case 1 (SFT) | 173 bp |
| Case 2 (SFT) | 165 bp |
| Case 3 (SFT/Hemangiopericytoma) | 160 bp |

Internal control: PGK1 (126 bp)

Courtesy of Mr. Long Jin

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### LIPOMATOUS TUMORS

- Extremely rare tumors in the orbit – 1% (AFIP)
- Few published series
- Mostly case reports
- Included in differential diagnosis with other lesions in the orbit
- Morphological classification validated by distinct chromosomal abnormalities


Font RL et al. AFIP Atlas of Tumor Pathology. 2006

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### Orbital Lipoma

- Orbital lipomas
- Spindle cell, pleomorphic types
- Differentiation with herniated orbital fat
- Benign lipomatous neoplasms (lipomas)
  - Majority display cytogenetic abnormalities
  - Aberrations involving 12q13-15


Tripathy D. Ophthal Plast Reconst Surg 2015;31:53

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### Orbital Liposarcoma

- Majority are myxoid or well-differentiated
- Dedifferentiated liposarcoma
- Exceedingly rare in the orbit

Al-Qahtani AA et al. Middle East Afr J Ophthalmol 2011; 18:314

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Font RL et al. AFIP Atlas of Tumor Pathology. 2006
Well-differentiated Liposarcoma

- MDM2 gene amplification
  - Also present in dedifferentiated liposarcoma
- MDM2 gene – located at 12q13-15
  - Encodes MDM2 protein
    - Negative regulator of p53 tumor suppressor
    - Overexpression – inactivates p53

Well-differentiated liposarcoma

- Amplification of MDM2
  - IHC, FISH, PCR, aCGH
- Co-amplification of genes in 12q14-15 region
  - CDK4, HMGA2, YEATS4, CPM and FRS2
  - Overexpression of MDM2 and CDK4 are not unique of liposarcoma

Liposarcoma – Well Differentiated

- MDM2 exon7 – TMPO exon4 fusion positive (145 bp)
Dedifferentiated Liposarcoma

- Co-amplifications involving 1p32 and 6q23
  - Includes JUN gene and its activating kinase ASK1
  - c-JUN pathway might be implicated in the progression to dedifferentiation

Myxoid Liposarcoma

- Majority cases - FUS-DDIT3(CHOP) fusion
  - T(12;16)(q13;p11)
  - 11 isoforms of fusion transcript
- Other cases – DDIT3(CHOP)-EWSR1 fusion
  - T(12;22)(q13;12)
  - 4 isoforms of fusion transcript
  - Different isoforms have not been implicated in differences in morphology or clinical outcomes

Ewing Sarcoma/ PNET

- Small round cell sarcoma
  - Varying degrees of neuroectodermal differentiation
  - Pathognomonic molecular findings
- Orbital ES/PNET exceedingly rare
  - Often included in differential diagnosis
Ewing Sarcoma/ PNET

- T(11;22)(q24;12) – common abnormality
- Balanced translocation – EWSR1 on chr22 and various members of ETS family of transcription factors
  - EWSR1-ETS fusion proteins activate or repress specific target genes – oncogenes
  - Key in pathogenesis of EWS/PNET

Ewing Sarcoma/ PNET

- T(11;22)(q24;12) – EWSR1-FLI1 fusion – 85%
- T(22;21)(q22;12) - EWSR1-ERG fusion
- Less common fusions
  - EWSR1-ETV1 fusion at 7p22
  - EWSR1-ETV4 fusion at 17q12
- Detection of fusions – improved diagnosis

EWSR1 by FISH

- BAP
- Normal

EWSR1-FL11

- P2 positive for EWSR1-FLI1
  - The fusion transcript is EWS1 exon 7 – FLI1 exon 6
- P1 is negative

Ewing Sarcoma/ PNET

- Prognosis associated with molecular alterations
  - EWSR1-FLI1 fusion – associated with longer disease-free survival
  - Alterations in p53 and p16/p14ARF (25% cases)
    - Aggressive clinical course
    - Refractory to chemotherapy

Mertens F et al. Semin Oncol 2009; 36:312
Lacrimal Gland Tumors

- Knowledge of behavior and their molecular pathogenesis – salivary gland tumor equivalent
- Most common types
  - Pleomorphic adenoma
  - Adenoid cystic carcinoma
  - Carcinoma ex-pleomorphic adenoma
  - Adenocarcinoma NOS

Pleomorphic Adenoma

- It represents 50% all epithelial tumors
- It occurs in the 5th and 6th decade
- No sex predilection
- Benign behavior
- Multiple recurrences if incompletely excised
- Potential to develop carcinoma

- 70% have cytogenetic abnormalities
  - Group 1 – 8q12 rearrangements – PLAG1
  - Group 2 – 12q13-15 rearrangements – HMGA2
  - Group 3 – non recurrent clonal changes (23%)
  - Group 4 – normal karyotype (30%)
  - PLAG1 IHC – positive in most PA

Pleomorphic Adenoma

- PLAG1- and HMGA2-containing fusion genes
  - Tumor specific
  - Diagnostic markers for PA
  - Detected by RT-PCR or FISH
  - High level expression of HMGA2 – malignant transformation of PA
45 year-old male presented in Oct ’14 with left eye motility problems, found to have also proptosis. Head CT revealed an orbital mass involving lacrimal gland, superior rectus muscle and levator complex.

Carcinoma Ex-Pleomorphic Adenoma
- Background pleomorphic adenoma with areas of malignancy – most common adenocarcinoma
- Alterations of p53 and p53 protein overexpression - malignant transformation
- Overexpression of c-erbB-2 and EGFR
  - Potential for target therapy
  - ICH biomarkers – AR, p53, HER-2/neu

Adenoid Cystic Carcinoma
- Biphasic tumor – ducts and myoepithelial cells
- Cribriform, tubular and solid pattern
- Slow-relentless biological behavior
- 75-80% - 5-year survival
- 35-20% - 15-year survival
- Solid growth – higher rate of node metastasis

Adenoid Cystic Carcinoma
- Recurrent t(6;9)(q22-23;p23-24)
- Results in MYB-NFIB fusion
  - Overexpression of MYB and downstream targets
  - Useful diagnostic biomarker
  - Potential therapeutic targets
- Other genomic alterations
  - Losses 1p, 6q, 12q and 17p
  - Gains ch22, 19q, 8q, and 11q

MYB - FISH
- BAP Normal
**Adenoid Cystic Carcinoma**
- Aggressive variant – ACC with high grade transformation
  - 50% rate of LN metastasis
  - p53 alterations in 40% cases
  - Gains in C-MYC gene locus (8q24)
  - Additional gains – 17q11.2-q12, 17q23 and 15q11-13

**Mucoepidermoid Carcinoma**
- 1-2% of all lacrimal gland neoplasms
- Mean age 49 yrs
- F:M ratio (3:2)
- Low and high-grade tumors

**Mucoepidermoid Carcinoma**
- Recurrent t(11;19)(q21-22;p13)
- Results in CRTC1-MAML2 fusion – 80% cases
  - Important diagnostic biomarker
  - Fusion-positive MEC – better prognosis
- HER2 and EGFR (60%) overexpression
  - High-grade tumors
  - Might have therapeutic relevance

**Summary**
- Biomarkers in a group of orbital tumors
  - Diagnostic purposes
  - Prognostic indicators
  - Potential target therapy

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