Malignant Pheochromocytoma (MP): A Comprehensive Genomic Profiling Study

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ABSTRACT

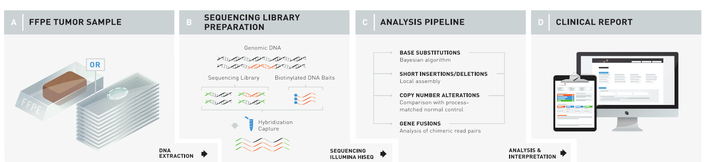
Background: We use CGP to characterize the genomic alterations (GA) in MP and to enable the search for potential therapy targets.

Methods: From a series of 201,766 consecutive clinical cases, 44 cases of clinically advanced MP underwent CGP using a hybrid-capture based commercial assay to evaluate all classes of GA. Tumor mutational burden (TMB) was determined on 0.83-1.14 Mbp of sequenced DNA and reported as mutations/Mb (mut/MB) and microsatellite instability (MSI) was determined on 114 loci. PD-L1 expression was determined by IHC (DAKO 22C3 antibody).

Results: All patients had clinically advanced recurrent and/or metastatic disease. 23 patients were females and 21 patients were males. There were 34 (77%) of MP known to have originated in the adrenal gland and 10 (23%) of the MP were sequenced from metastatic site where the exact primary site was unknown. The primary tumor was used for sequencing in 14 (32%) of the MP cases and a non-primary tumor metastatic site (liver, lung, bone, soft tissue, lymph node, kidney, peritoneal cavity, and chest wall) in 30 (68%) of the MP cases. There were 2.3 GA/tumor. The most frequent un-targetable GA were *ATRX* (25%), *TP53* (21%), *SDHB* (13%), *CTNNB1* (7%), VHL (7%), and *CDKN2A/2B*, *PIK3R2*, *NOTCH2* and *MEN1* (all 5%). The most frequent potentially targetable GA included *RET* (9%), *NF1* (9%) and *FGFR1* (5%). *PBRM1* GA were found in 2% of MAP. Germline mutations in known cancer predisposition genes were predicted in 8 (18%) of cases involving *SDHB* (5 cases) and *BRCA1*, *MEN1*, and *MSH2* (1 case each). The genomic signatures of primary MP were not significantly different from that obtained from sequencing of metastatic site biopsies. 0 (0%) of 5 MP stained positively for PD-L1 expression. The mean TMB was 2.95 mutations/Mb, the median TMB was 2.4 mutations/Mb. There 2 (5%) of MP with TMB ≥ 10 mutations/MB and 0 (0%) with TMB ≥ 20 mutations/Mb. 0 (0%) of 33 MP evaluated for MSI had a MSI-High status.

Conclusions: Although the GA/tumor is relatively low for MP, CGP can reveal important potential therapy targets including *RET*, *NF1* and *FGFR1*. MP do not reveal strong potential for immunotherapies with low TMB, absence of MSI-High status and low (2%) *PBRM1* mutation frequencies.

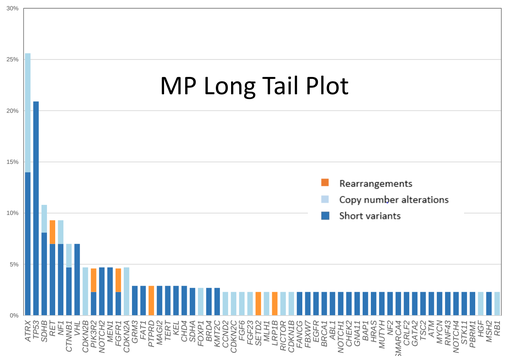
MATERIALS AND METHODS

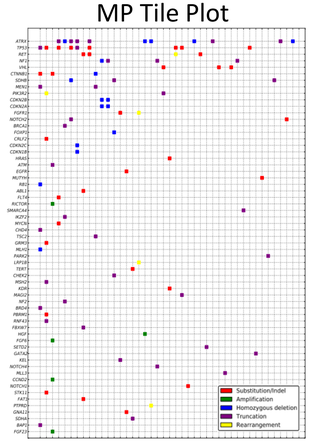


- ≥50 ng DNA extracted from 40 µm of FFPE sections
- Sequencing performed for up to 315 cancer-related genes and introns from 28 genes commonly rearranged in cancer
- Hybrid capture-based sequencing using adaptor ligation-based libraries
- Mean coverage depth >600X
- Base substitutions, insertions and deletions (short variants; SV), rearrangements, and copy number changes were assessed
- Tumor mutational burden (TMB) calculated from 0.83-1.14Mb sequenced DNA

RESULTS

- 23 patients were females and 21 patients were males and all (100%) had advanced/metastatic disease
- Primary site was the adrenal gland for 34 (77%) and unknown for 10 (23%)
- Primary tumor sequenced in 14 (32%) and metastatic site in 30 (68%): No difference in GA based on whether primary site or metastatic site was used for sequencing
- There were 2.3 GA/tumor.
- Most frequent un-targetable GA were *ATRX* (25%), *TP53* (21%), *SDHB* (13%), *CTNNB1* (7%), VHL (7%), and *CDKN2A/2B*, *PIK3R2*, 1 *NOTCH2* and *MEN1* (all 5%)
- Most frequent potentially targetable GA included *RET* (9%), *NF1* (9%) and *FGFR1* (5%)
- *PBRM1* GA in 2% of MAP
- Germline mutations in known cancer predisposition genes predicted in 8 (18%) of cases involving *SDHB* (5 cases) and *BRCA1*, *MEN1*, and *MSH2* (1 case each)
- Mean TMB was 2.95 mut/Mb, the median TMB was 2.4 mut/Mb, 2 (5%) had TMB ≥ 10 mutations/MB and 0 (0%) and TMB ≥ 20 mut/Mb
- No MP evaluated (0/33) for MSI had a MSI-High status.
- No MP (0/5) stained positively for PD-L1 expression

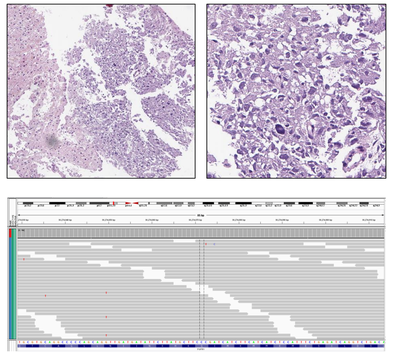




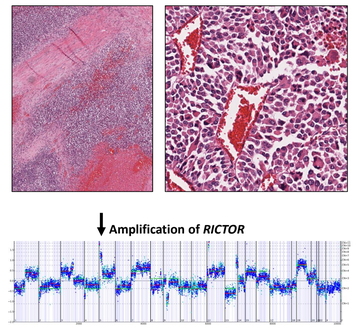
CONCLUSIONS

- MP represent a rare form of endocrine cancer that feature a variety of genomic alterations
- Although the GA/tumor is relatively low for MP, CGP can reveal important potential targets for therapy in the metastatic setting including *RET*, *NF1* and *FGFR1*
- MP do not reveal strong potential for immunotherapy with low TMB, absence of MSI-High status and low (2%) *PBRM1* mutation frequencies
- Based on this data, further study of CGP as a method of developing precision therapies for MP appears warranted

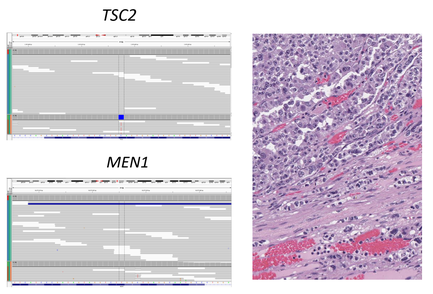
CASES



Metastatic adrenal pheochromocytoma to the liver in a 57 year old Latino female. The only genomic alteration in this sporadic pheochromocytoma was an activating mutation in the *FGFR1* gene (N546K). The tumor was MSI stable and had a Tumor Mutation Burden of 4 mut/MB of sequenced DNA. *FGFR1* plays key roles in regulation of the cell cycle and angiogenesis and is an upstream regulator of the RAS, MAPK, and AKT signaling pathways. *FGFR1* mutations were reported in one of 398 pheochromocytomas but not in 15 paragangliomas analyzed in the COSMIC dataset (Aug 2018). FGFR1 has been reported to be overexpressed in pheochromocytoma tissues when compared with normal medulla conferring a greater risk of tumor metastasis. Tumors with alterations that activate *FGFR1* may be sensitive to FGFR family inhibitors including the multikinase inhibitors pazopanib and ponatinib. Other inhibitors of FGFR1, such as AZD4547, BGJ398, Debio 1347, INCB054828, JNJ-42756493, TAS-120, and the multikinase inhibitors lenvatinib and lucitanib, are under clinical investigation.



Sporadic malignant pheochromocytoma in a 45 year old Caucasian man. Comprehensive genomic profiling of the primary tumor revealed amplification of the *RICTOR*, *CCND2, FGF23* and *FGF6* genes along with short variant mutations in *ATM* L2211fs*4 and *CTNNB1* S45P. *RICTOR* (rapamycin-insensitive companion of mTOR, or raptor-independent companion of mTOR) encodes an mTOR-binding protein that is part of the mTORC2 complex. *RICTOR* amplification has been detected in 0.6% (1/159) of cases in the Pheochromocytoma and Paraganglioma TCGA dataset and in 7% (6/92) of cases In the Adrenocortical Carcinoma TCGA dataset (cBioPortal, Dec 2014). Tumors with *RICTOR* amplification may be sensitive to inhibitors of mTORC2. Numerous inhibitors that target both mTORC1 and mTORC2 complexes, as well as dual PI3K/mTOR inhibitors, are under preclinical and clinical investigation in multiple tumor types. Preclinical studies have explored combined mTORC1 and mTORC2 targeting as potential therapeutic strategy against pheochromocytomas.



Malignant pheochromocytoma of the left adrenal in a 71 year old African American woman. The primary tumor was 8 cm, featured focal necrosis and lymphovascular invasion. The PD-L1 IHC stain was negative. Comprehensive genomic profiling revealed short variant mutations in *TSC2* Q166*, *CTNNB1* loss exons 2-3 and *MEN1* splice site 928-9_942del24. The *MEN1* mutation in this patient had a mutant allele frequency and was consistent with a germline mutation. Although not a frequent tumor in the classic MEN1 familial cancer syndrome, germline mutations in *MEN1* are a recognized feature in a small subset of malignant pheochromocytomas. Loss of function mutations in *TSC2* are associated with activation of mTOR and may predict sensitivity to mTOR inhibitors. The mTOR inhibitors everolimus and temsirolimus are FDA approved and other mTOR inhibitors and dual inhibitors of PI3K and mTOR are under investigation in clinical trials in many solid tumor types.