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Chromosome 3p loss of heterozygosity and reduced H3K36me3 correlate with longer relapse free survival in sacral conventional chordoma

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Abstract

Conventional chordoma is a rare slow-growing malignant tumor of notochordal origin primarily arising at base of skull and sacrococcygeal bones. Chordoma may arise from its benign counterpart, benign notochordal cell tumors, and can also undergo dedifferentiation progressing into dedifferentiated chordoma. No study has directly compared the genomic alterations among these tumors comprising a morphologic continuum. Our prior study identified frequent chromosome 3p loss of heterozygosity and minimum deleted regions on chromosome 3 encompassing *SETD2*, encoding a histone methyltransferase involved in the trimethylation of lysine-36 of histone H3 (H3K36me3). In the current study we expanded our study to include 65 sacral conventional chordoma cases, 3 benign notochordal cell tumors and 2 dedifferentiated chordomas using SNP array, targeted Next-Generation Sequencing analysis and immunohistochemistry. We performed immunohistochemical analysis of histone, H3K36me3 and investigated whether there is any association between the clinical behavior and recurrent

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chromosome or aneuploidy, or H3K36me3 protein expression. We found that there is increased genomic instability from benign notochordal cell tumor to conventional chordoma, to dedifferentiated chordoma. The highly recurrent genomic aberration, chromosome 3p loss of heterozygosity (occurred in 70% of conventional chordomas), is correlated with longer relapse free survival, but not with overall survival or metastasis free survival in sacral chordoma. Chordomas demonstrate variable patterns and levels of H3K36me3 expression and reduced expression of H3K36me3 showed marginally significant correlation with longer relapse free survival. Copy number alterations in the genes encoding H3K36me3 methylation transferase complex and demethylase may account for the altered H3K36me3 expression levels.

Introduction

Conventional chordoma is a rare slow-growing malignant tumor of notochordal origin primarily arising at base of skull and sacrococcygeal bones (WHO 2013). Histologically, chordoma cells are arranged in lobules containing ribbons and cords with hallmark “physaliphorous” cytoplasm in an abundant myxoid matrix. Nuclei can show varying degrees of pleomorphism and mitotic figures are infrequent in general. Dedifferentiated chordoma is a biphasic tumor exhibiting areas of conventional chordoma juxtaposed to a high-grade sarcoma devoid of notochordal features. Prognostically, conventional chordoma has overall median survival of 7 years and up to 40% noncranial chordomas metastasize to lung, bone and lymph nodes (WHO 2013), while dedifferentiated chordoma has a worse prognosis most patients die within one year of diagnosis [1].

The benign counterpart, benign notochordal cell tumors (BNCTs), show “adipocyte-like” cells with well-defined cell borders and absence of cytologic atypia or extracellular matrix. Most BNCTs are incidental findings with similar anatomic site distribution to chordoma. The risk of transformation to a chordoma is considered to be extremely low. Although the exact relationship between the two entities and the underlying genomic alterations are unclear, BNCT juxtaposed to a chordoma has been noted in a few studies, suggesting that chordomas may arise from progressive transformation of their benign counterparts [2-4].

In cases where there is juxtaposition of BNCT and chordoma, increase in cellularity and myxoid changes are regarded as signs of malignant transformation. The progression from BNCT-chordoma-dedifferentiated chordoma could thus be construed as a morphologic continuum associated with genomic complexity. However, no study has directly compared the genomic alterations among these tumors.

Trimethylation of lysine 36 on histone 3 (H3K36me3) is a histone posttranslational modification enriched at gene bodies of transcriptionally active genes [5, 6]. It is involved in diverse cellular processes including epigenetic regulation of gene transcription, mRNA splicing, DNA damage repair and cell cycle regulation etc., In humans, methylation of H3K36 is catalyzed by several methyltransferases including NSD1 and NSD2, which catalyze H3K36me1/2, and SETD2, which catalyzes H3K36me1/2/3 [5]. Demethylation of H3K36 is catalyzed by JmjC-domain containing enzymes, KDM4A-C [7]. The dysregulation of H3K36 methylation through mutations of lysine residues of histone H3 proteins, methyltransferases or demethylase is found to be associated with a number of

human neoplasms, such as clear cell renal cell carcinoma, chondroblastoma, acute leukemia, and head and neck squamous cell carcinoma (SqCC) [8-12]. Our prior study identified frequent chromosome 3p loss of heterozygosity (LOH) and two minimum deleted regions (MRDs) on chromosome 3 encompassing cancer associated chromatin remodeling and putative tumor suppressor genes, *SETD2*, *BAP1*, and *PBRM1* [13]. *SETD2* was also found to harbor inactivating mutation in addition to LOH in a few cases, making it a potential target tumor suppressor gene (TSG) on chromosome 3p. In the current study we expanded our study to include 65 sacral conventional chordoma cases, 3 BNCTs and 2 dedifferentiated chordomas using SNP array and targeted Next-Generation Sequencing analysis. We also performed H3K36me3 immunohistochemistry (IHC) and investigated whether there is any association between the clinical behavior (overall survival (OS), relapse free survival (RFS) and metastasis free survival (MFS)) and recurrent chromosome or arm level copy number alterations (aneuploidy), or H3K36me3 protein expression.

Methods and Materials

Patient Data and Tumor Specimens

This study was approved by institutional review board 17-067 and 12-245, and was conducted in accordance with the U.S. Common Rule. Patients with diagnosis of chordoma and adequate tumor material were selected from 1993 to 2017. The follow-up data were available up to about 213 months (more than 17 years). The H&E slides were reviewed by 3 pathologists (one subspecialized bone and soft tissue pathologist) and tumors with adequate tumor content (majority > 70%) are subjected to SNP array or NGS-targeted sequencing analysis. Clinical data were obtained through manual review of electronic medical record.

OS, RFS and MFS definitions: OS: measured by the time from the initial diagnosis to cancer related death; RFS: measured by time to relapse (TTR): the time from surgical resection to local relapse, or the time from the initial diagnosis to the appearance of distant metastasis, whichever comes first; metastasis free survival (MFS): measured by time to metastasis (TTM), the time from the initial diagnosis to the appearance of distant metastasis.

A total of 45 cases were subject to copy number analysis by SNP assay (34 cases) or NGS-targeted sequencing (11 cases) platforms. This includes previously published 23 cases[13].

SNP array analysis

Genomic DNA was extracted from frozen (n=20) and formalin-fixed and paraffin-embedded (FFPE, n=14) tumor tissues. Genome-wide DNA copy number alterations and allelic imbalances were analyzed by SNP-array using Affymetrix OncoScan Assay (Affymetrix, CA) using eighty (80) nanograms of genomic DNA for each sample. Processing of samples was performed according to the manufacturer's guidelines (Affymetrix). OncoScan SNP-array data were analyzed by the software couple of OncoScan Console (Affymetrix) and Nexus Express (BioDiscovery, CA) using Affymetrix TuScan algorithm. All SNP array data were also manually reviewed for subtle alterations not automatically called.

NGS-targeted Sequencing by MSK-IMPACT

Copy number information in eleven cases were analyzed on the MSK-IMPACT platform [14, 15] a validated custom hybridization capture-based assay, which is capable of detecting somatic mutations, small insertions and deletions, copy number alterations and selected structural rearrangements. Three different versions were used in this study containing 341, 410 or 468 genes. Only CNA of chromosome 3p information from these 11 cases were used for this study. Detailed technical information can be found in the corresponding references [14, 15]. Briefly, copy number aberrations were identified by comparing sequence coverage of targeted regions in a tumor sample relative to a standard diploid normal sample. Fold change < -1.5 is considered deletion of chromosome 3p.

Immunohistochemical Staining

The immunohistochemical staining for H3K36me3 was performed at the Department of Pathology, Memorial Sloan-Kettering Cancer Center using a commercially available antibody, clone MAB10333 (61021; Active Motif, Carlsbad, CA) at 1:500 dilution (0.88 $\mu\text{g/mL}$). Staining was performed on the Leica Bond-3 auto staining system (Leica, Buffalo Grove, IL), using heat-based antigen retrieval, a high pH buffer solution (AR9640; Leica, Bond Epitope Retrieval Solution 2, 30 minutes), 30-minute primary incubation time, and a polymer detection system (DS9800; Leica, Bond Polymer Refine Detection).

Semi-quantification of H3K36me3 IHC intensity using modified H-score

The H3K36me3 staining intensities in the tumor cell nuclei were scored as 0, 1, 2, or 3 corresponding to the presence of negative, weak, intermediate, and strong staining, respectively. The percentages of cells with positive staining were assessed. An increment of 10% between 0 and 100% was used for quantifying the percentage of positive tumor cells. The average positive percentage value, H-score, for every case was calculated as following: $\text{H-score} = \% \text{ of cells with positive staining} \times \text{average staining intensity (0, 1, 2 or 3)}$. An H-score between 0 and 300 was obtained where 300 was equal to 100% of tumor cells stained strongly (3+).

Results

Clinical and pathologic features

In total, 70 notochordal tumors arising from the sacrococcygeal (n=69) or lumbar (n=1) spine were included in the current study. They consist of 3 benign notochordal cell tumors with areas of early transformation to chordoma (BNCT/early chordoma), 65 conventional chordomas and 2 dedifferentiated chordomas with associated conventional chordoma. The patients include 22 females and 48 males with a median age of 57 (range of 23-85 years) at primary diagnosis. Distant metastases developed in 24% (16/67) of patients. The metastatic sites include lung, other bones, soft tissue, liver, and lymph node (one case). Local recurrence occurred in 34% (23/67) of patients. Among the 64 patients whose surgery or radiation therapy (RT) data are available, gross total resection of the primary tumor was achieved in 62 cases (97%), for which 66% (41/62) achieved negative resection margin. Fifteen of the sixty-four patients (23%) received preoperative RT on the primary tumor, 17%

(11/64) received postoperative RT and 59% (38/64) never received any RT for their primary tumors. Among 41 patients with negative margins, 23 did not receive RT on their primary tumor, 11 had preoperative RT, 6 had postoperative RT, and in one patient RT information was not available. The sizes of the primary tumors (including BNCT) ranged from 1.4 cm to 40 cm in greatest dimension (median: 7 cm). The summary of the clinical information is included in table 1.

Increased genomic instability from benign notochordal cell tumor to conventional chordoma, to dedifferentiated chordoma

Three sacrococcygeal benign notochordal cell tumors (with areas of early transformation to chordoma), thirty sacrococcygeal conventional chordomas, and two sacrococcygeal dedifferentiated chordomas arising from chordoma were subject to genome-wide high-resolution SNP-array analysis. All three BNCT/early chordomas showed normal diploid genome without large-scale copy number alterations (CNA) or allelic imbalance (Figure 1). All the conventional chordomas except one had large-scale CNA and/or copy-neutral loss of heterozygosity (CN-LOH) involving at least one chromosome. The median number of chromosomes being involved is 9 (range: 0-18). The dedifferentiated components from the two dedifferentiated chordomas had large-scale CNA and/or CN-LOH involving almost all the chromosomes (20 and 22 chromosomes respectively). Their conventional chordomas components had four and eleven chromosomes being involved by large-scale CNA and/or CN-LOH, respectively. In summary, the paired conventional chordoma to dedifferentiated chordoma cases showed great increases in the number of chromosomes involved by large-scale CNAs in these two cases. (One representative pair shown in Figure 1).

Chromosome 3p loss of heterozygosity is a highly recurrent genomic aberration in sacral chordoma

Similar to our previous reported findings [13], recurrent genomic alterations are dominated by large-scale deletion or CN-LOH, which frequently involves chromosome 3p (70%), 3q (70%), 9p (53%), 1p (57%), 14 (43%), 10 (53%), and 13 (43%). Gain of chromosome 7 (47%) is the only highly recurrent copy number gain (suppl. Figure 1). Among the SNP array cohort (n=30, not including BNCT and dedifferentiated chordoma) of sacrococcygeal conventional chordoma, 70% (21/30) of tumors had copy number loss, or CN-LOH involving at least part of chromosome 3p (Figure 2). Sixteen cases had heterozygous deletion of nearly the entire 3p (53%). One case had complete homozygous deletion of the entire 3p (CD36) and one case had CN-LOH of entire 3p (CD13). One case had both 3p CN-LOH and focal deletion (CD9). Two cases had deletion of a middle segment of 3p (CD1, CD19). Among the 21 cases with 3p aberration, 85.7% (18/21) also had concurrent deletion or CN-LOH of chromosome 3q (Figure 2). The minimal deleted region encompasses three critical tumor suppressor genes, *SETD2*, *BAP1* and *PBRM1*, as previously reported where 3p LOH was found in 63% of cases [13].

Chromosome 3p loss of heterozygosity is correlated with longer RFS, but not with OS or MFS in sacral chordoma

In addition to the 34 SNP array cases, CNA information of chromosome 3p was extracted from 11 cases of MSK-IMPACT sequencing data to increase the number of cases with

clinical follow-up data. Of 45 cases altogether, only the patients whose primary conventional chordoma were treated with gross total resection and had informative follow-up data are used in Kaplan-Meier survival analyses. Totally, survival analyses were performed on 36 patients based on their OS, MFS, RFS and chromosome 3p CNA status. Chromosome 3p loss of heterozygosity (either by deletion or CN-LOH) is significantly associated with longer RFS ($p=0.026$), but not with either OS ($p=0.98$) or MFS ($p=0.46$) (Figure 3). The median RFS time for 3p-LOH group is 92 months in contrast to 36 months in 3p-normal group. RFS is not significantly different between margin-positive and margin-negative groups for the cases that chromosome 3p status are known ($p=0.26$). OS, MFS and RFS Kaplan-Meier survival analyses were also performed based on chromosome 7 gain or not, and primary tumor sizes. However, no statistically significant associations were found between these parameters and survival times (data not shown).

H3K36me3 expression pattern and intensity differ among sacral chordomas

H3K36me3 IHC was performed on 3 BNCT/early chordomas and 53 conventional chordomas. All three BNCT/early chordomas showed strong and diffuse nuclear signal of H3K36me3 antibody (Figure 4). However, this was not the case with conventional chordomas, where the staining pattern and intensity were variable. Four major patterns were observed: diffuse and strong, focal loss, mosaic pattern, and near complete loss (Figure 4). Semi-quantification of H3K36me3 staining intensity of each case was calculated using modified H-score [16], which is based on both the intensity and number of positive cells ($\text{H-score} = \% \text{ of cells with positive staining} \times \text{average staining intensity}$, see Methods and Materials). The median H3K36me3 H-score was 240 (range: 30-300).

Reduced H3K36me3 expression shows marginally significant correlation with longer RFS

Clinical follow-up data was available for 45 patients who underwent gross total resection of their primary tumors (BNCT/early chordoma cases were not included). OS, MFS and RFS Kaplan-Meier survival analyses were performed based on H3K36me3 H-score, which is divided into two groups: low level ($\text{H-score} \leq 240$) or high level ($\text{H-score} > 240$). Patients with low H3K36me3 level showed a marginally significant association with longer RFS ($p=0.059$) compared to patients with high level, but not with either OS ($p=0.12$) or MFS ($p=0.32$) (Figure 5). The median RFS time for low H3K36me3 group is greater than 277 months (the median is indeterminate since less than 50% cases developed relapse) in contrast to 90 months in high H3K36me3 group. BNCT with early transformed tumors were excluded.

H3K36me3 expression intensity correlates with copy number alterations in the genes encoding H3K36me3 methylation transferase complex and demethylase

A total H3K36me3 CNA scores (see Methods and Material for calculation method) were calculated for each case by adding up all the gene scores. The H3K36me3 CNA scores was plotted against the H3K36me3 H-scores for 24 tumors for which both SNP array data and H3K36me3 IHC were performed. H3K36me3 H-scores and CNA scores showed a weak but significant positive linear correlation ($r=0.32$, $p=0.005$, $R=0.32$) (Figure 6).

Discussion

Chordoma is a slow-growing malignant bone tumor with metastatic potential. In contrast, BNCTs are indolent benign tumors and only rarely transform to chordoma. Dedifferentiated chordoma is a more aggressive neoplasm, and has a higher rate of metastasis than conventional chordoma. Morphologically, increased cellularity, nuclear atypia and myxoid changes signifies the progression from BNCTs to conventional chordoma and further transformation to a dedifferentiated chordoma requires a phenotypic switch to non-notochordal high grade sarcoma. These phenotypic changes are associated with increased genomic complexity reflected by the median number of chromosomes involved in the large-scale CNA/CN-LOH (0, 9 and 21 respectively) assessed in this study. Our finding is consistent with previous studies in other tumors where morphological features of increased cytologic atypia correlated significantly with multiple measures of genomic complexity such as number of whole genome doublings and aneuploidy [17]. Thus assessment of nuclear features including size, shape, variability, and chromatin quality etc., which is a critical parameter in tumor grading used by pathologists for hundreds of years, is an indirect measurement of the tumor's genomic complexity.

This study and previous studies have shown that conventional chordoma is characterized predominantly by copy-number changes with low mutational loads and few recurrently mutated genes [13, 18], which is similar to the findings in other types of adult sarcomas [17]. The CNA in chordomas is mainly composed of large-scale deletion or CN-LOH (chromosome 3, 9p, 1p, 14, and 13) with small number of chromosome or arm gain (chromosome 7 and 1q). The whole chromosome or arm imbalance, namely aneuploidy, vary across tumor types and are often unique to certain types of cancer. For instance, chromosome arm 8p is frequently lost in breast cancers and lung adenocarcinomas, but is commonly gained in seminoma [19]. Complete or partial loss of chromosome arm 3p, seen in 70% of the chordomas in the current study, are also frequently observed in esophageal SqCC, head and neck SqCC, lung SqCC, cholangiocarcinoma and clear cell renal cell carcinoma [19]. About 86% of chordomas with 3p loss in this study also had concurrent loss of chromosome arm 3q, not reported in the aforementioned cancers where more commonly there is gain or no alteration in 3q, suggesting cancer type specific selection in the pattern of aneuploidy.

The minimal deleted region on 3p includes three known tumor suppressor genes, *SETD2*, *BAP1* and *PBRM1*, with no inactivating mutation or homozygous deletion found in the majority of cases [13, 18]. The high recurrence of loss of 3p and absence of “second hit” in any known tumor suppressor gene on 3p in most cases suggest a compound haplo-insufficiency among the tumor suppressor genes (loss of one tumor suppressor gene allele is functionally significant only after compound loss of other genes). Myelodysplastic syndrome (MDS) with 5q deletion, monosomy 7 or 7q deletion provide prototypical examples of human cancer associated with compound haplo-insufficiency. No recurrent inactivating mutations in the minimal deletion region of 5q, 7 or 7q have been identified. Recapitulation of human MDS phenotype in engineered mouse models with equivalent 5q deletion further lend support for the compound haplo-insufficiency in promoting tumorigenesis [20, 21]. Therefore, 3p deletion likely confers chordoma growth

advantage through compound haplo-insufficiency of tumor suppressor genes on 3p, which in combination with unique aneuploid pattern (such as deletion of 3q, 9p, 1p, 14, 10, and 13 as well as gain of 7) drives chordoma tumorigenesis. Numerous studies have provided evidence that co-occurrence of certain aneuploidies through genomic evolution promotes tumor development under the right circumstances of tumor stage, cell type, genetic make-up, tumor microenvironment and immune system [22].

Degree and pattern of aneuploidy can also provide prognostic value. For instance, high levels of aneuploidy or CNA are associated with worse disease-free and overall survival in breast, endometrial, clear cell renal cell, thyroid, and colorectal cancer [9, 23]. In keeping with these earlier studies, we found in this study that there is increased genomic complexity from BNCTs to conventional chordoma to dedifferentiated chordoma, which have poorer prognosis in order. In terms of single chromosome or arm level aneuploidy, monosomy 5 or 5q deletion alone is associated with good OS in MDS patients [24, 25] while in glioma co-deletion of 1p/19q of oligodendroglioma is associated with lower rate of relapse and longer overall survival than other types of gliomas [26]. In this study, we found 3p deletion is correlated with longer RFS, but not with OS or MFS. Interestingly, deletion of 3p through genome engineering in human lung cells induces slowed cell cycle and slower initial cellular proliferation [19], which sheds some light on the biological basis accounting for longer RFS in chordoma allowing for future functional study. Thus 3p LOH could be potentially useful for predicting chordoma prognosis.

Based on our previous observation on abnormal H3K36me3 level in some chordomas, we further investigated its expression pattern by immunohistochemistry in 53 chordomas and 3 BNCT/early chordomas in this study. Compared to its consistent strong and diffuse intensity in BNCT/early chordomas, the staining pattern of H3K36me3 was variable among conventional chordomas and can be summarized as four patterns: near complete loss, focal loss, mosaic, and strong & diffuse. The H3K36me3 intensity is positively correlated with total genes dosage that are involved in catalyzing H3K36 methylation and demethylation, suggesting the deregulation of H3K36me3 level might be a result of CNA in the genes maintaining this histone posttranslational marker. Studies from recent years have demonstrated the mechanisms of how deregulation of H3K36 methylation, as result of H3K36M mutation or methyltransferase inactivation, leads to cancer development. For instance, the histone mutation H3K36M observed in chondroblastoma could induce undifferentiated sarcomas as result of genome-wide gain in H3K27 methylation and mesenchymal cell differentiation blockage because of global reduction of H3K36 methylation caused by inhibition of methyltransferase enzymatic activity by mutation H3K36M [11, 27]. Similarly, inactivation of NSD1 found in subset of head and neck squamous cell carcinoma, or NSD1 fusion observed in acute myeloid leukemia drives cancer formation through a common mechanism involving epigenome deregulation [12, 28].

In this study, we also observed that reduced H3K36me3 expression show a marginal correlation with longer RFS, which is in keeping with correlation of 3p loss with longer RFS. Previous studies have also used altered H3K36 level or mutation status in genes maintaining H3K36 methylation to predict prognosis. For example, loss of histone

H3 lysine 36 trimethylation is found to be associated with an increased risk of renal cell carcinoma-specific death [29], while increased H3K36 trimethylation predicts poor prognosis in hepatocellular carcinoma [30]. SETD2 mutation was associated with worse cancer-specific survival and is more likely to present with advanced disease in clear cell renal cell carcinoma [31, 32]. Low SETD2 expression was associated with reduced overall and progression-free survival in metastatic renal cell carcinoma [33] and correlated with development of metastasis, local recurrence and death in breast cancer [34]. Our findings of altered H3K36me3 expression in chordomas likely reflects its functional status and could be indicative of tumor behavior in addition to loss of chromosome 3p.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Representative H&E stain and SNP array from sacral benign notochordal tumor (A), conventional chordoma (B) and dedifferentiated chordoma (C). The SNP array shows increase in genomic abnormalities from A to B to C.

Figure 2.

Chromosome 3p is recurrently deleted in sacral conventional chondroma. Red color: deletion; yellow: allele imbalance. MRD: Minimal Deletion Region. 70% (21/30) of tumors had copy number loss, or CN-LOH involving at least part of chromosome 3p. Sixteen cases had heterozygous deletion of nearly entire 3p (53%). One case had homozygous deletion of 3p (CD36) and one case had CN-LOH of 3p (CD13). One case had both 3p CN-LOH and focal deletion (CD9). Two cases had deletion of middle segment of 3p (CD1, CD19). Among the 21 cases, 85.7% (18/21) also had concurrent deletion or CN-LOH of chromosome 3q. The minimal deleted region encompasses three critical tumor suppressor genes, *SETD2*, *BAP1* and *PBRM1*.

Figure 3.

Chromosome 3p loss of heterozygosity correlates with longer RFS, but not with OS or MFS.

Figure 4. H&E and H3K36me3 immunohistochemistry of sacral benign notochordal cell tumor and conventional chordoma. H3K36me3 expression pattern and intensity differ among sacral chordomas. Four major patterns were observed, diffuse and strong, focal loss, mosaic intensity and near complete loss.

Figure 5. Reduced H3K36me3 expression show marginally significant correlation with longer RFS, but not with OS and MFS in sacral conventional chordoma.

Figure 6.
H3K36me3 expression levels correlate with copy number alterations in the genes encoding H3K36me3 methylation transferase complex and demethylase.

Table 1.

Summary of Clinical Features.

	All cases	Cases included in the K-M analysis			
		SNP+IMPACT (n=36 [*])		IHC (n=45)	
	Total	3p normal	3p loss	H3K36me3 high	H3K36me3 low
Number of patients	70	11	25	24	21
Female / Male	22/48	2/9	12/13	6/18	6/15
Median age at 1° Dx (year)	57 (23-85)	54	61	58	55
Radiation ^{**}	preop: 23% (15/64) postop: 17% (11/64)	preop: 45% (5/11) postop: 9% (1/11)	preop: 8% (2/24) postop: 25% (6/24)	preop: 21% (5/24) postop: 21% (5/24)	preop: 25% (5/20) postop: 15% (3/20)
1° tumor size (cm)	7 (1.4 - 40)	8.5 (5-13)	8.5 (4.3-25.4)	8.5 (1.5-40)	7 (2.9-25)
Negative margin ^{**}	66% (41/62)	78% (7/9)	64% (14/22)	74% (17/23)	55% (11/20)
Percent relapse (local+met) ^{**}	36% (23/64)	55% (6/11)	56% (14/25)	58% (14/24)	29% (6/21)
Percent metastasis ^{**}	25% (16/64)	18% (2/11)	32% (8/25)	38% (9/24)	24% (5/21)
Percent death at the end of f/u ^{**}	26% (17/66)	27% (3/11)	40% (10/25)	42% (10/24)	19% (4/21)
Median Overall Survival (m)	207	>183	209	209	180
Median Time To Relapse (m)	101	37	92 p=0.026	90	>277 p=0.059
Median Time To Metastasis (m)	>279	>181	184	184	>277

^{*}Only the patients whose primary tumor were treated with gross total resection and had informative follow-up data are used in Kaplan-Meier survival analyses. Totally, survival analyses were performed on 36 patients based on their OS, MFS, RFS and chromosome 3p CNA status.

^{**}The clinical information (radiation, margin, relapse, metastasis, death) were not available for all the cases. The denominator indicates the actual number of cases in which corresponding clinical information is available.