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Paternal Uniparental Disomy with Segmental Loss of Heterozygosity of Chromosome 11 are Hallmark Characteristics of Syndromic and Sporadic Embryonal Rhabdomyosarcoma

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Abstract

Costello syndrome (CS) arises from a typically paternally derived germline mutation in the proto-oncogene *HRAS,* and is considered a rasopathy. CS results in failure-to-thrive, intellectual disabilities, short stature, coarse facial features, skeletal abnormalities, congenital heart disease, and a predisposition for cancer, most commonly embryonal rhabdomyosarcoma (ERMS). The goal of this study was to characterize CS ERMS at the molecular level and to determine how divergent it is from sporadic ERMS. We characterized eleven ERMS tumors from eight unrelated CS patients, carrying paternally derived *HRAS* c.34G>A (p.Gly12Ser; 6) or c.35G>C (p.Gly12Ala; 2) mutations. Loss of heterozygosity (LOH) was evaluated in all CS ERMS by microarray and/or short tandem repeat (STR) markers spanning the entire chromosome 11. Eight CS ERMS tumors displayed complete paternal uniparental disomy of chromosome 11 (pUPD11), whereas two displayed UPD only at 11p and a second primary ERMS tumor showed UPD limited to 11p15.5, the classical hallmark for ERMS. Three sporadic ERMS cell lines (RD, Rh36, Rh18) and eight formalin fixed paraffin embedded (FFPE) ERMS tumors were also analyzed for RAS mutations and LOH status. We found a higher than anticipated frequency of RAS mutations (*HRAS* or *NRAS*; 50%) in sporadic ERMS cell lines/tumors. Unexpectedly, complete uniparental disomy (UPD11) was observed in five specimens, while the other six showed LOH extending across the p and q arms of chromosome 11. In this study, we are able to clearly demonstrate complete UPD11 in both syndromic and sporadic ERMS.

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Keywords

Costello Syndrome; embryonal rhabdomyosarcoma; loss of heterozygosity; uniparental disomy; chromosome 11; RAS mutations; 11p15.5

INTRODUCTION

The goal of this study was to characterize the molecular signatures of embryonal rhabdomyosarcoma in Costello syndrome patients and determine how it compared to that of nonsyndromic cases of ERMS. Costello syndrome (CS, OMIM #218040) is a rare congenital disorder estimated to affect about 1/500,000 newborns worldwide [Gripp and Lin, 2012]. CS affects multiple organ systems and has a wide range of clinical presentations. CS patients have failure-to-thrive, severe postnatal feeding difficulties, short stature, and share characteristic distinctive facial features including sparse, curly or fine hair, full nasal bridge, thick lips, and epicanthal folds [Gripp and Lin, 2012; Siegel et al., 2012]. Skin abnormalities include papillomas, hyperpigmentation, hyperkeratosis, and loose, soft, and redundant skin [Gripp and Lin, 2012; Siegel et al., 2012].

CS is caused by a heterozygous germline mutation in the proto-oncogene *HRAS,* arising in most cases in the paternal germline [Aoki et al., 2005; Gripp et al., 2006; Sol-Church et al., 2006; Zampino et al., 2007]. *HRAS* is located at 11p15.5, and most mutations are found in codons 12 and 13, with p.Gly12Ser in more than 80% [Aoki et al., 2005; Gripp, 2005; Gripp et al., 2006]. These gain-of-functions mutations prevent accessibility for GTPase activating proteins (GAPs) to exchange GTP for GDP, thus RAS remains constitutively active leading to increase cell proliferation, differentiation, apoptosis, and cell mobility [Shields et al., 2000]. Patients with Costello syndrome have a higher susceptibility than the general population to develop benign and malignant tumors, with an incidence of 15% [Gripp, 2005; Gripp et al., 2006; Kerr et al., 2009]. The most common solid tumors in Costello syndrome patients are rhabdomyosarcomas [Gripp and Lin, 2012]. Rhabdomyosarcoma (RMS) is the most prevalent sporadic pediatric soft tissue sarcoma with an incidence of 4.5 cases per million children each year [Ries et al., 1999]. In the United States an estimated 350 children are diagnosed with sporadic RMS each year [Huh et al., 2010]. Embryonal rhabdomyosarcoma (ERMS) is the most common subtype, accounting for over 60% [Belyea et al., 2012]. A recent review from the Children's Oncology Group reported a 5-year event free survival rate in rhabdomyosarcoma clinical groups I/II and III of 69% and 70%, respectively [Wolden et al., 2015]. Though no long term follow up is available at this time for our CS cohort, 10 (77%) of the 13 individuals who underwent therapy survived while three (23%) died due to rhabdomyosarcoma tumor progression or relapse. One recently enrolled patient is currently undergoing treatment. Limitations in our data include grouping all individuals and rhabdomyosarcoma types together, the limited information on tumor stage and treatment protocols as well as lack of long term follow up data. While acknowledging the small Costello syndrome cohort size and limited data, we conclude that there is no obvious difference in survival after rhabdomyosarcoma treatment compared to a larger cohort of presumably nonsyndromic rhabdomyosarcoma.

One hallmark of sporadic ERMS is uniparental disomy (UPD) at 11p15.5, a region that contains *HRAS* as well as a cluster of imprinted genes [Visser et al., 1997; Anderson et al., 1999]. RAS mutations have been reported in 5–35% of sporadic ERMS [Stratton et al., 1989; Chen et al., 2006; Kratz et al., 2007]. Of the 141 patients with a germline *HRAS* mutation currently enrolled in our study, fourteen developed embryonal rhabdomyosarcoma (ERMS), from which eight primary tumors as well as three relapsed tumor specimens from eight unrelated patients were used to study mutation and LOH status. The CS tumors were analyzed in parallel with specimens derived from sporadic ERMS, which allowed us to demonstrate complete uniparental disomy beyond what has been previously reported around 11p15.5.

MATERIAL AND METHODS

Biological Specimens

CS patients and families were identified through physician referral and enrolled in an ongoing research study approved by the Institutional Review Board of the A.I. duPont Hospital for Children. Biological specimens included saliva and blood from the CS probands and available family members, as well as fresh or FFPE ERMS tumor samples. A cell line was established using routine tissue culture protocols from one Costello syndrome patient (CS 242). Additional ERMS cell lines from sporadic cases were either purchased from ATCC (RD ERMS) or received through a generous gift from Dr. Peter Houghton from Nationwide Children's Hospital (Rh18 and Rh36). Eight deidentified FFPE sporadic ERMS tumor samples were obtained from the Alfred I. duPont Hospital for Children Biobank. For two of these ERMS specimens matched non neoplastic stoma tissue was available.

DNA Isolation

Genomic DNA was extracted from all specimens (blood, saliva, cells, tumor) using the PureGene DNA Isolation Kit (Qiagen). The DNA Isolation Kit was used with the following modifications from the manufacturer's protocol to isolate DNA from FFPE samples: three 300μl xylene rinses with a five minute incubation followed by three 300μl 100% ethanol washes with a five minute incubation. The samples were then processed through cell lysis buffer as stated in the protocol.

RAS PCR sequencing

Samples were processed for Sanger sequencing by the COBRE-funded Nemours Biomolecular Core using standard workflows and operating procedures. *HRAS* was sequenced as previously described [Sol-Church et al., 2009]. Primers for NRAS 5′-TGAGGGACAAACCAGATAGGCA-3′ and 5-′ TGGTTCCAAGTCATTCCCAGTAG-3′ were annealed at 55°C to amplify a discrete 571 bp fragment.

Short Tandem Repeats (STRs) Analysis

Molecular confirmation that CS probands genetically matched their parents was completed using the AmpFISTR Profiler Plus amplification kit and/or Identifiler PCR amplification kit (ThermoFisher, Grand Island, NY). For LOH analyses of Chromosome 11, ABI LMS V2 Ch11 markers were used as previously described in Gripp et al., [2006].

Cytoscan Analysis

The Affymetrix Cytogenetics GeneChip Cytoscan HD arrays were used as recommended by the manufacturer. The data were analyzed using the Affymetrix chromosome analysis suite software.

Metaphase prep and Fluorescence *in situ* hybridization (FISH)

Metaphase prep for cell culture was modified from the preparation of metaphase chromosomes of adherent cells (NCI National Institute of Health, 2006). All incubations were for 15 mins and all centrifugations were performed for 8 mins at 350xg. First, 300μl of Actinomycin D (AG Scientific, San Diego, CA) at 100μg/mL was added to the flask and incubated for 15mins. Next, 300μl KaryoMax® Colecmid® Solution (Gibco® Life Technologies, Grand Island, NY) was added to the flask. FISH protocols were adapted from Coriell. The centromeric probe used was CEP 11 (D11Z1) SpectrumGreen probe 11p11.11-q11 Alpha Satellite DNA (06J37-021 Abbott Molecular, Abbot Park, IL). In addition, the slides were stained with ProLong gold antifade reagent with DAPI (P36935 Molecular Probes Life Technologies Grand Island, NY) and stored at −20°C. Images were taken at 63x on a Leica CTR microscope using Perkin Elmer Volocity 6.1.1.

RESULTS

Tumor and Cell Specimens

Prior to molecular analysis, fresh tumor specimens were placed in cultures and fixed specimens were embedded in paraffin and processed for hematoxylin & eosin (H&E) staining by the Nemours' Histotechnology Core, to assess tissue integrity. Histological assessments were made by pathologists and the deidentified Biobank's tumor paraffin blocks were confirmed to contain tumor cells (at least 80%) prior to sectioning and DNA extraction. Though tumor histology varied from patient to patient, typical findings included small cells, primitive spindle-shaped cells, myofibrils and cross striations on light microscopy after H&E stain. A classic example of bladder botryoid ERMS is presented for patient CS 187. Note the morphologic features resembling a cluster of grapes (Figure 1A), as well as ERMS characteristic histological findings of cross striations and rhabdomyoblastic differentiation (Figure 1B). Table I includes clinical features of the eight unrelated CS patients. Eleven CS-ERMS tumor specimens were obtained from these patients (Table II). We were unable to determine if the CS 170 tumor procurement was prior to or after treatment. However, we have pre-chemotherapy tumor tissues from proband's CS 242, CS 457, CS 393, CS 214-T2, and CS 283-T1. We have post-chemotherapy tumor tissues from CS 181 and CS 187-T2. We have both pre and post chemotherapy tumor tissue from CS 187-T1 and CS 214-T1. In addition to the eight primary tumors, two metastatic specimens were obtained from CS 187 and CS 283. The metastatic diagnosis in these patients was based on the time course as well as location of the tumors. CS 214 developed two ERMS tumors eleven years apart that were localized in different body regions; CS 214-T1 was an abdominal ERMS while CS 214-T2 was paratesticular, thus these tumors were clinically diagnosed as two primary tumors. The two CS 214 tumors have distinct molecular signatures as seen by STR analysis (see section below) which may indicate they arose independently and thus support the clinical assessment of a second primary ERMS. A pure ERMS cell line was established from a fresh

biopsy obtained post-surgery from CS 242. There have been no previous reports studying ERMS tumorigenesis using a cell line derived from a patient with Costello syndrome. Attempts to establish pure cell lines from the other two fresh biopsies failed due to specimen size and lack of viable tumor myoblasts. In addition to the CS specimens, three sporadic ERMS cell lines (RD, Rh18, Rh36), and eight sporadic ERMS FFPE tumors were included in the study for comparative analysis.

Mutation Status

PCR amplification was performed to screen specimens derived from Costello syndrome patients for mutations in all exons of *HRAS*. In addition to the tumor samples, biospecimens included all eight CS proband's blood, buccal and/or fibroblast tissues, and respective samples from their unaffected parents. A CS diagnosis was confirmed in each patient through the presence of a heterozygous germline *HRAS* mutation: c.34G>A (p.Gly12Ser) in six cases and c.35G>C (p.Gly12Ala) in two (Table I). Using allelic specific amplification, the mutation was found to be inherited through the paternal germline in six families. In CS 214 and CS 457, no informative SNPs were found near *HRAS*, paternal origin of the mutation was inferred using STR analysis as described below. Figure 2 is a classic example of how chromatograms were used to determine the *HRAS* mutation status for CS 242. This patient carries a c.35G>C heterozygous germline mutation in buccal, skin, and skin fibroblast cells. Both unaffected parents carry the wild-type allele. The patient's ERMS-derived cell line displays the homozygous mutation indicating complete loss of the wild-type allele. All CS tumors showed loss of the wild type *HRAS* allele (Table II). In specimens harboring low levels of stromal contamination (CS 214-T1, CS 187-T1, CS 181, CS 170, CS 283-T1/T2) the wild-type allele was detectable. Interestingly, in addition to the homozygous p.Gly12Ser mutation, CS 187 tumor specimens (primary and metastatic) also carry a heterozygous c.202C>T (p.Arg68Trp) mutation that is absent from the germline.

In the eleven sporadic tumor specimens, a mutation screen was performed on *HRAS*, *KRAS*, *NRAS*, and *ERAS*. Surprisingly, six of the eleven specimens carried a gain of function mutation in either *HRAS* or *NRAS* (Table II). Even more striking, sporadic tumors from patients P666 and P664 harbor homozygous *HRAS* mutations, that were absent from germline.

Loss of Heterozygosity

In embryonal tumors, the most common chromosomal aberration is loss of heterozygosity (LOH) at 11p15.5. To investigate the status of allelic loss at 11p15.5, 18 short tandem repeat (STR) markers were used that span the p and q arms of chromosome 11. Figure 3 demonstrates the use of STR to identify complete uniparental disomy as well as parental origin in CS 242 ERMS tumor cell line. Only two loci (11p and 11q) are presented here but the loss extended to all 18 markers on chromosome 11. Comparing the CS profile to that of the parent, one can clearly see at both loci complete loss of the maternal allele is observed in the tumor and the tumor derived ERMS cell line. All but three CS tumors showed paternal uniparental disomy (paternal-UPD, pUPD11) with complete loss of the maternal allele across the entire chromosome 11. The first exception was CS 214-T2 which displayed the classically described LOH uniquely at 11p15.5 that is referred to as uniparental disomy of

11p15.5. The other two exceptions (CS 393 and CS 457) displayed segmental paternal uniparental disomy on the entire 11p arm. Unexpectedly, all sporadic tumors showed segmental loss of heterozygosity beyond the expected 11p15.5 locus. Furthermore, complete uniparental disomy of the whole chromosome 11 was identified not only in RD and Rh36 ERMS cells but also in FFPE biopsies obtained from other patients with sporadic ERMS (P670, P664, P646). This demonstrates that complete uniparental disomy for the whole chromosome 11 in sporadic tumor-derived cell lines is not an artifact of cell culture but normal tumor progression in sporadic ERMS. Our results demonstrate for the first time that the majority of CS and sporadic ERMS tumors display complete loss of one parental chromosome 11 allele (uniparental disomy for the whole chromosome 11). One tumor showed the hallmark of ERMS with uniparental disomy at the 11p15.5 locus and two displayed segmental uniparental disomy localized to the entire 11p arm (11pUPD). Strikingly, this study found that the majority of CS ERMS tumors have lost the maternal allele which we may infer to be similar in the sporadic tumors.

Cytogenetic analysis

This STR study was complemented by Cytoscan analysis, a microarray technique enabling the detection of chromosomal aberrations such as LOH and copy number variation. This array analysis identified typical gains and losses in portions of chromosomes 2, 7, 8, 11, 12, 13, 17, 19, and 20, and confirmed LOH on chromosome 11 p and q (data not shown). Fluorescence *in situ* hybridization (FISH) was performed using a centromere chromosome 11 probe on CS 242 ERMS and fibroblasts, and sporadic ERMS cell lines (RD, Rh18). As depicted in Figure S1, CS 242 fibroblasts display two centromere 11 probes in 100% of cells. In contrast, ERMS heterogeneity was revealed through FISH as disomic and trisomic centromere 11 probes were detected. Thus, monosomy was excluded as a cause of the LOH in these CS and sporadic ERMS cases. Our data from Cytoscan and FISH suggest that UPD11 in the disomic or trisomic state is common in CS and sporadic ERMS.

DISCUSSION

We enrolled, through the Nemours Rasopathy program, the most extensive cohort of Costello syndrome patients and their family members. Of the 141 individuals affected with Costello syndrome 138 carry a germline heterozygous mutation in *HRAS* while 3 individuals displayed somatic mosaicism [Gripp et al., 2006; Sol-Church et al., 2009]. In our cohort 16 individuals (13%) developed malignancies (ERMS, neuroblastoma, bladder carcinoma), in support of CS as a cancer predisposition syndrome [Gripp, 2005; Kerr et al., 2006]. ERMS was identified in 14 individuals, and 11 different tumors from eight unrelated CS patients were analyzed in this study. Neuroblastoma developed in one patient with a p.Gly12Ala and one patient with a p.Gly12Ser. Likewise, transitional cell bladder carcinoma was identified in two patients with either a p.Gly12Ala or a p.Gly12Ser. Since ERMS is the most prevalent cancer observed in our CS patients, the focus of this study was to better understand ERMS tumorigenesis not only in the context of our syndromic model, Costello syndrome, but additionally in sporadic ERMS where mutations in one of the RAS genes is reported in 14%–35% [Stratton et al., 1989; Chen et al., 2006; Kratz et al., 2007; Shern et al., 2014]. As expected, all CS tumors displayed loss of the WT maternal HRAS allele

which is consistent with the classical paternal uniparental disomy at 11p15.5 previously reported in ERMS. In one instance (CS 187) the tumor acquired a second variant, a heterozygous c.202C>T (p.Arg68Trp) mutation. This patient relapsed due to metastasis carrying the same heterozygous variant. It is unclear whether the p.Arg68Trp mutation contributed to the aggressive nature of this tumor resulting in the death of this patient. While the mutation is found in the switch II domain and may result in constitutively active RAS/MAPK signaling, there have been no other cancers reported with this mutation, and it is not reported in the COSMIC database.

In sporadic tumors, we identified RAS mutations in 54% of cases, with six of eleven sporadic ERMS tumors carrying a mutation in either *HRAS* or *NRAS.* Two of these sporadic tumors, P666 and P664, carried a homozygous *HRAS* mutation, p.Gly12Cys and p.Gly13Arg, respectively, which are absent in their matched non-tumor FFPE sample. Interestingly, of the five p.Gly12Cys CS patients enrolled in our cohort, only one developed ERMS. The p.Gly13Arg variant is not observed in our CS cohort, however, it is a very common mutation seen in the heterozygous state in nevus sebaceous, a benign congenital skin lesion [Groesser et al., 2012]. Since these FFPE samples were deidentified to use as sporadic controls, no clinical data are available. The codons most frequently associated with cancer in KRAS, NRAS, and HRAS are 12, 13, and 61. Therefore, it is not surprising to see mutations in codons 12, 13, and 61 when screening ERMS tumors. Though our sample size is low, it is possible that previous studies on ERMS underreported the number of RAS mutations, perhaps due to incomplete screening or tumor heterogeneity.

Loss of heterozygosity (LOH) and the classically described 11p15.5 uniparental disomy (UPD) have been reported as the major hallmark of ERMS [Visser et al., 1997; Kratz et al., 2007; Menke et al., 2015]. Our data confirmed LOH at chromosome 11 in all ERMS specimens and revealed widespread allelic loss along the length of chromosome 11 with segmental uniparental disomy of 11 occurring in two CS and six sporadic tumors. This segmental uniparental disomy of 11 was not only localized to 11p, but it was found in the q arm in several of the sporadic ERMS tumors. It is striking that complete uniparental disomy of the entire chromosome 11 was observed in 13 of the 22 ERMS specimens, with five occurring in sporadic ERMS cell lines and FFPE tumors. Complete loss of heterozygosity in the sporadic FFPE tumor specimens confirms that loss of a parental chromosome 11 allele is not an artifact of cell culture, but rather a common mechanism of tumor progression. LOH at 11p15.5 in sporadic ERMS is associated with loss of the maternal allele [Scrable et al., 1989]. We have shown in all eight CS ERMS patients that the maternal wild-type allele was lost, which supports previous studies on sporadic ERMS cases. Therefore, we speculate that there is loss of the maternal allele in the eleven sporadic ERMS specimens.

A previous study of seven orbital ERMS tumors using chromosome 11 microsatellite markers showed that two markers 11q13.1-22.3 and 11q23 displayed LOH [Mastraneglo et al., 1998]. However, complete UPD was not observed in these tumors. Patient CS 214 developed an abdominal primary tumor that was procured pre and post-chemotherapy treatment. Both tumors showed the same STR profiles indicating complete paternal uniparental disomy of the entire chromosome 11. The patient developed a second primary paratesticular tumor that may have been detected in its early stage due to regular monitoring

or the tumor location. This second primary tumor interestingly has a different signature (STR profile) with cells harboring uniquely the classical 11p15.5 uniparental disomy. It is tempting to suggest that loss of heterozygosity at the 11p15.5 locus may be a prerequisite for ERMS formation. Complete uniparental disomy of the entire chromosome 11 more likely arose from the loss of the maternal chromosome in the CS and sporadic tumors via trisomic rescue [Tuna et al., 2009, Lapunzina et al., 2011] with or without the need of the 11p15.5 UPD. Figure 4 presents a model for ERMS tumorigenesis, updated from Kratz et al [2006], that is derived from the STR profile heterogeneity found in the CS and sporadic ERMS in this study. This model includes the classically described uniparental disomy of 11p15.5, as well as, segmental uniparental disomy of chromosome 11 that both occur through mitotic recombination events, and the complete parental uniparental disomy of the entire chromosome 11 observed in the majority of our tumor specimens that results via trisomic rescue as a result of mitotic nondisjunction events. These unexpected widespread findings of segmental and complete uniparental disomy of 11 are reminiscent of what has been described in about 20% of Beckwith-Wiedemann syndrome (BWS) patients that have variable segmental disomy in the 11p region. A few BWS cases have shown complete parental uniparental disomy of chromosome 11 [Cooper et al., 2007]. Despite that both BWS and CS patients are prone to developing ERMS, BWS patients display an overgrowth phenotype while CS patients have a short stature and failure-to-thrive.

In summary, our study of sporadic and syndromic ERMS specimens reveals features not reported before. Firstly, we suggest that the mutation load in sporadic tumors may have been underestimated and that RAS mutations are common in these cancers. Secondly, we propose that the classical 11p15.5 uniparental disomy may not entirely characterize ERMS. Indeed most specimens, whether isolated from a patient with Costello syndrome or from sporadic cases, show complete loss of one parental chromosome 11.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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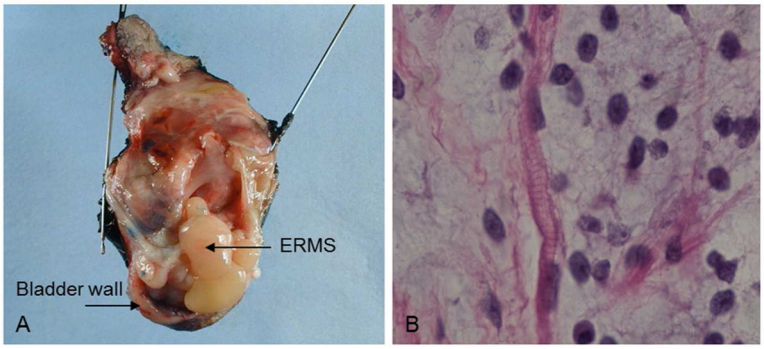

Figure 1.

Costello Syndrome embryonal rhabdomyosarcoma tumor (A) Photographic image of CS patient 187 resected bladder; this ERMS tumor infiltrates the bladder and prostate; (B) This ERMS section with hematoxylin and eosin staining reveals rhabdomyoblastic differentiation and cross striations identified by the pathologist. [Print in black and white]

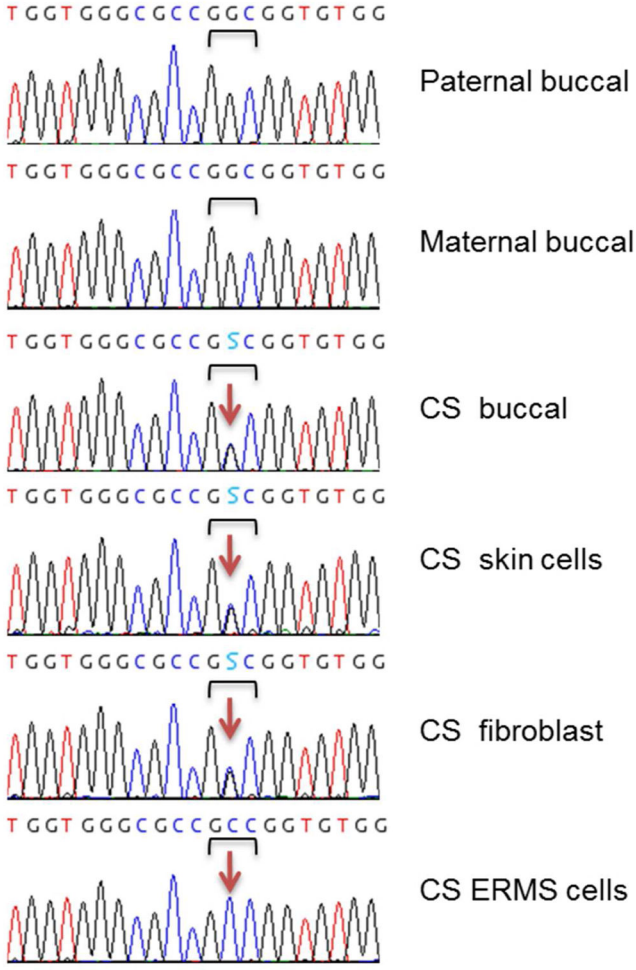

Figure 2.

Loss of the *HRAS* wild-type allele in Costello Syndrome embryonal rhabdomyosarcoma. Chromatograms show the germline c.35 G>C (p.Gly12Ala) *HRAS* mutation in CS patient 242. The bracket indicates the Gly12 triplet while the arrow indicates the mutation site. The S in the sequence (C or G) indicates the actual heterozygous mutation. The proband's ERMS cells display complete loss of the wild-type allele (G). [Print in black and white]

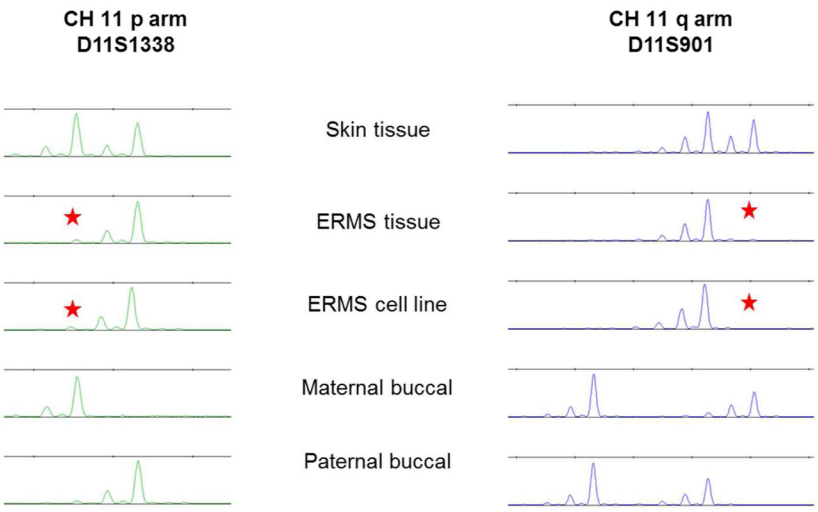

Figure 3.

Chromosome 11 loss of heterozygosity in Costello Syndrome embryonal rhabdomyosarcoma. Chromosome 11 STR (short tandem repeats) markers reveal loss of heterozygosity in Costello Syndrome ERMS. This electropherogram is a representative profile of two of the eighteen STR loci on the p and q arms of chromosome 11 (D11S1338 and D11S901) that shows LOH of the maternal allele in the tumor tissue and cell line. The star indicates loss of the maternal wild-type allele. [Print in black and white]

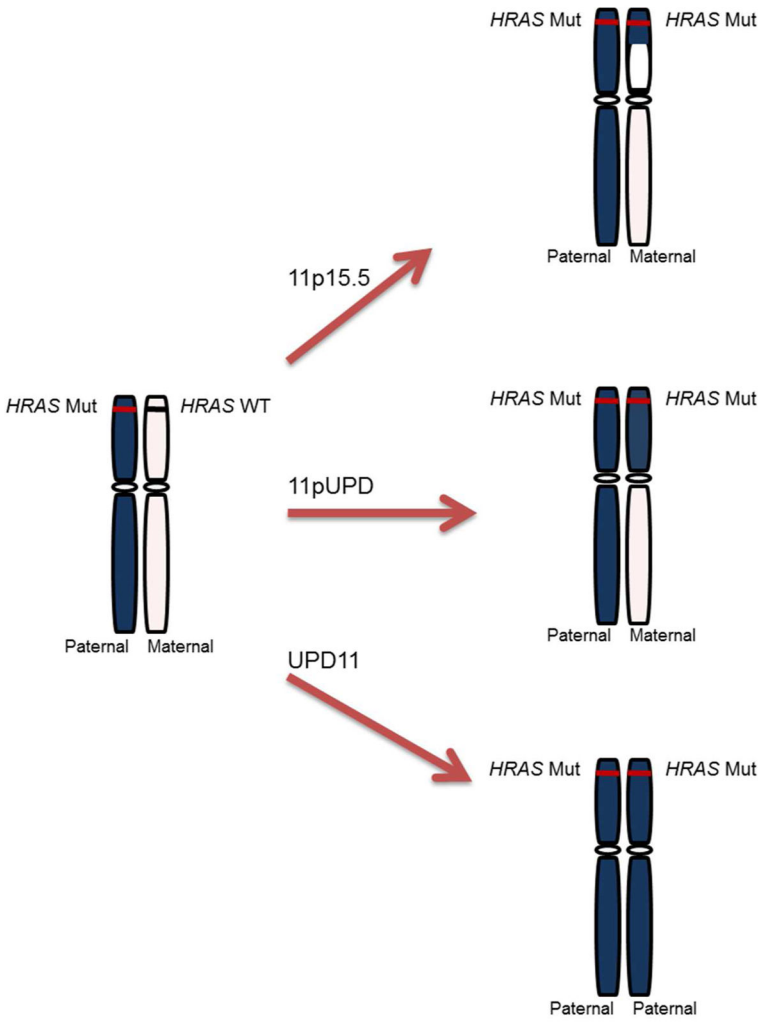

Figure 4.

Proposed model for tumorigenesis in Costello Syndrome and sporadic embryonal rhabdomyosarcoma tumors. We characterized uniparental disomy in our ERMS tumors three ways, 1) the classically described uniparental disomy at 11p15.5, 2) segmental uniparental disomy of chromosome 11 which includes uniparental disomy of the whole P arm (11pUPD), 3) complete uniparental disomy of the entire chromosome 11 (UPD11) or paternal uniparental disomy of the whole chromosome 11 (pUPD11). First, the classically described uniparental disomy only at 11p15.5 was only found in 1 tumor in our study. Next, segmental uniparental disomy of 11 was found in 8 out of 22 ERMS cases. The most commonly observed molecular ERMS characteristic in this study was paternal uniparental disomy for the whole chromosome 11 (pUPD11) or complete uniparental disomy for the whole chromosome 11 (UPD11) in our CS and sporadic tumors. The mechanism of action of uniparental disomy at 11p15.5 or segmental uniparental disomy of 11(11pUPD) can be explained through mitotic recombination events, and complete parental uniparental disomy of the whole chromosome 11 (UPD11) and uniparental disomy for the whole chromosome 11 (pUPD11) can be explained through trisomic rescue. [Print in black and white]

Table I

Costello Syndrome Cohort

Patient ID	Gender	Germline Mutation and Additional Molecular Findings	Mutation Origin	Age ERMS Diagnosed (Years)	Location	Tumor Histology	Source of Tumor Sample	Timing of Sample Procurement	Notes
CS 170[a]	F	*HRAS* p.Gly12Ser	Paternal	2.75	Pelvic	ERMS	FFPE	N/A	Alive and well
CS 181[a]	M	*HRAS* p.Gly12Ser FISH analysis revealed 15–19% of cells are XXY; 10–16% of cells have gains in chromosome 13 (3–5 copies) and chromosome 21 (3 copies)	Paternal	16	Pelvic involving bladder, prostate	ERMS with botryoid features	Fresh Tissue	Post-treatment	Deceased
CS 187[a]	M	*HRAS* p.Gly12Ser	Paternal	1.5	Bladder	ERMS	Fresh Tissue	T1-Pre/Post treatment T2-Post-treatment of primary tumor	Pulmonary/Thoracic metastatic ERMS at 2.9; deceased
CS 214[a]	M	*HRAS* p.Gly12Ser	Paternal	1.5	Abdominal Paratesticular	Mixed ERMS and ARMS ERMS	FFPE	T1-Pre & Post-treatment T2-at diagnosis, pre-treatment	Paratesticular second primary ERMS at age 13; Alive and well
CS 242[a,b]	F	*HRAS* p.Gly12Ala	Paternal	1.7	Abdominal/pelvic	ERMS	Fresh Tissue Cell line	Pre-treatment	Deceased
CS 283[a,b]	M	*HRAS* p.Gly12Ala	Paternal	2	Sphenoid	ERMS, spindle cell variant	FFPE	T1-Pre treatment T2-diagnosis of recurrence	Nasopharyngeal ERMS recurrence at age 4; Alive and well
CS 457	M	*HRAS* p.Gly12Ser	Paternal	1.3	Seminal Vesicle	ERMS	Fresh Tissue	Pre-treatment	Completed treatment, alive and well
CS 393	F	*HRAS* p.Gly12Ser	Paternal	3	Retroperitoneal	ERMS	Fresh Tissue	Pre-treatment	In treatment

Abbreviations are as follows: ID, identification; ERMS, embryonal rhabdomyosarcoma; F, female; M, male; FISH, fluorescence in situ hybridization; FFPE, formalin fixed paraffin embedded; N/A, not available; ARMS alveolar rhabdomyosarcoma.

a and b additional clinical findings reported can be found respectively in references Gripp et al., 2010; Detweiler et al., 2013.

Table II

Genomic status of embryonal rhabdomyosarcoma

Proband	ERMS Location	Source of Tumor Sample	Timing of Sample Procurement	Mutation Status	LOH Status
CS 170	Pelvis	FFPE	N/A	*HRAS* Hom p.Gly12Ser	Paternal UPD11
CS 181	Pelvic involving bladder, prostate	Fresh Tissue	Post-treatment	*HRAS* Hom p.Gly12Ser	Paternal UPD11
CS 187-T1	Bladder	Fresh tissue	Pre/Post treatment	*HRAS* Hom p.Gly12Ser; HRAS Het p.Arg68Trp	Paternal UPD11
CS 187-T2	Thoracic	Fresh tissue	Post-treatment	*HRAS* Hom p.Gly12Ser; HRAS Het p.Arg68Trp	Paternal UPD11
CS 214-T1	Abdominal	FFPE	Pre/Post-treatment	*HRAS* Hom p.Gly12Ser	Paternal UPD11
CS 214-T2	Paratesticular	FFPE	Pre-treatment	*HRAS* Hom p.Gly12Ser	11p15.5 UPD
CS 242	Abdominal/pelvic	Fresh tissue, and Cell line	Pre-treatment	*HRAS* Hom p.Gly12Ala	Paternal UPD11
CS 283-T1	Sphenoid	FFPE	Pre-treatment	*HRAS* Hom p.Gly12Ala	Paternal UPD11
CS 283-T2	Nasopharyngeal	FFPE	Diagnosis of reoccurrence	*HRAS* Hom p.Gly12Ala	Paternal UPD11
CS 457	Seminal Vesicle	Fresh tissue	Pre-treatment	*HRAS* Hom p.Gly12Ser	11p UPD
CS 393	Retroperitoneal	Fresh tissue	Pre-treatment	*HRAS* Hom p.Gly12Ser	11p UPD
RD	Pelvis	Cell line	N/A	*NRAS* Het p.Gln61His *TP53* Het p.Trp248	UPD11
Rh36	Paratesticular	Cell line	N/A	*HRAS* Het p.Gln61Lys	UPD11
Rh18	Perineum	Cell line	N/A	N/D	Partial LOH
P673	N/A	FFPE	N/A	*NRAS* Het p.Gln61Lys	Partial LOH
P666	N/A	FFPE	N/A	*HRAS* Hom p.Cys12	Partial LOH
P664	N/A	FFPE	N/A	*HRAS* Hom p.Arg13	UPD11
P657	N/A	FFPE	N/A	*NRAS* Het p.Gln61Lys	Partial LOH
P670	N/A	FFPE	N/A	N/D	UPD11
P643	N/A	FFPE	N/A	N/D	Partial LOH
P646	N/A	FFPE	N/A	N/D	UPD11
P662	N/A	FFPE	N/A	N/D	Partial LOH

Abbreviations are as follows: FFPE, formalin fixed paraffin embedded; Hom, Homozygous; Het, Heterozygous; LOH, loss of heterozygosity; UPD, uniparental disomy; N/A, not available; N/D, not detected.