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Occurrence and Summary of Literature Documenting Tumors with *EWSR1* and *CREB* Family Transcription Factor Fusions

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Abstract

Background: Fusions between the *EWSR1* gene and *CREB* family transcription factors have been reported in a variety of rare tumor types, including the head and neck tumors clear cell odontogenic carcinoma (CCOC) and clear cell carcinoma (CCC) of the salivary gland.

Objective: To review the current literature on tumors where *EWSR1-CREB1* fusions have been reported (i.e., *EWSR1-ATF1*, *-CREB1*, *-CREM*) that may act as surrogates for clear cell odontogenic carcinoma (CCOC) and clear cell carcinoma (CCC) of the salivary gland given their rarity.

Methods: The PubMed database was searched for tumors bearing *EWSR1* and *CREB* family transcription factor fusions. The search was conducted independently by two authors between the publication dates of 1985 and 2021. Search parameters included but were not limited to "*EWSR1-ATF1*," "*EWSR1-CREB11*," and "*EWSR1-CREM*." Data collected included tumor name, *EWSR1* translocation partner, histology, immunohistochemical (IHC) markers, presence of clear cells, patient demographics and anatomic location when available.

Results: A total of 103 articles were reviewed and 17 different types of tumors (CCOC and CCC included) were identified. The following 6 were found to consistently bear the same translocations characteristic of CCOC and CCC: angiomatoid fIbrous histiocytoma (AFH), clear cell sarcoma (CCS), clear cell sarcoma-like tumor of the gastrointestinal tract (CCSLTGT), intracranial myxoid mesenchymal tumor (IMMT), malignant gastrointestinal neuroectodermal tumor (M-GNET), and primary pulmonary myxoid sarcoma (PPMS). The remaining 9 tumors were either inconsistently identified or rarely harbored an *EWSR1-CREB1* fusion: angiosarcoma, atypical central neurocytoma, malignant epithelioid tumor of the peritoneal cavity, malignant mesothelioma, neuroendocrine neoplasm, primary intracranial neoplasm, pulmonary mesenchymal tumor, small blue round cell tumor and soft tissue myoepithelial tumor.

Conclusion: *EWSR1-CREB1* fusions have been reported in many tumor types and our review of the literature shows that these fusions are a consistent finding in a smaller set of 6 diagnostic entities in addition to CCOC and CCC. While these tumors exhibit differences in demographics, anatomic location and immunohistochemical profiles, the high frequency of *EWSR1-CREB1* fusions suggests that they may be driven by similar oncogenic mechanisms. Identifying similarities among these rare tumor types may thus be advantageous for gaining insights into their pathogenesis.

Keywords: CCOC; CCS; CCC; *EWSR1*; *CREB1*; *CREM*; *ATF1*; fusion

Introduction

Ewing sarcoma breakpoint region 1 (*EWSR1*) is a gene on chromosome band 22q12.2 that encodes an RNA-binding protein and was first identified as a partner in fusions in Ewing sarcoma [1]. The N-terminal disordered domain is responsible for the protein's transcriptional activation role in the context of gene fusions [2]. Since their initial identification, *EWSR1* translocations have been observed in a variety of tumors and has been shown to have several different fusion partners. In fact, Ewing sarcoma is often characterized by the presence of a gene fusion between *EWSR1* and members of the *ETS* family including *FLI1*, *ERG* and *FEV* [3-5]. To date, most tumors with an *EWSR1* translocation are sarcomas originating in mesenchymal tissues, with the only known exceptions being CCOC (clear cell odontogenic carcinoma) and CCC (clear cell carcinoma of the salivary gland) [6,7].

The National Cancer Institute defines a rare tumor as a cancer that occurs in fewer than 15 out of 100,000 people each year. Most rare cancers are often more difficult to prevent, diagnose, and treat than the more common ones. The study of rare tumors is difficult due to the lack of availability of fresh tissue. As such, to increase the cohort of cases, surrogates need to be identified based on commonalities in clinical behavior, histopathology, IHC and molecular and genetic transcriptional defects. Our tumor of interest is clear cell odontogenic carcinoma (CCOC) and has only 119 published cases worldwide (1985-2021). Additionally, its intraosseous nature results in many of these specimens having been decalcified. It is also an extremely slow

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growing tumor making it difficult to grow in the laboratory. A similar tumor, clear cell carcinoma of the salivary gland (CCC, formerly known as hyalinizing clear cell carcinoma), is postulated to be its soft tissue counterpart and is almost as rare with a total of 254 cases reported in the literature [6-8].

Both CCC and CCOC demonstrate comparable clinical behavior, have similar IHC patterns and have translocations involving the *EWSR1* gene with translocation partners of *ATF1*, *CREB1* or *CREM* [7,9]. Eight different teams [6,9-15] have studied the genetics of CCOC. To date, twenty COCC specimens have been tested for *EWSR1* translocation using either fluorescence *in situ* hybridization (FISH) [6,13,15] or RT-PCR and FISH analysis [11], or RNA-seq [9,14]. *EWSR1* is shown to be partnered with *ATF1* (n=7), *CREB1* (n=2), *CREM* (n=2) [9,14] and, the remainder with an unknown rearrangement location and/or partner. Six of these specimens, however, were negative for an *EWSR1* translocation [6,10]. The significance of this is unknown but may reflect limitations of the sequencing technologies used.

Both CCC and CCOC have a preponderance of clear cells [8,16] although the significance of these clear cells is unknown. clear cell sarcoma (CCS) may also be similar to CCOC and CCC. All three tumor types have clear cells and a *EWSR1* rearrangement involving partners in the *CREB* family of transcription factors (*ATF*1, *CREB1* or *CREM*). Additionally, these tumors appear in soft tissue as well as bone, have a prolonged clinical course and appear to have a slight predilection to women [8,16].

In this study, tumors with the same gene fusions that are found in CCOC (i.e., *EWSR1-ATF1*, *-CREB1*, *-CREM*) were identified and summarized.

Materials and Methods

The databases available through PubMed were used to identify articles describing *EWSR1-CREB1* family of transcription factors fusion-harboring tumors. The PubMed database was searched independently by two authors between the publication dates of 1985 and 2021. Search parameters included but were not limited to "*EWSR1-ATF1*," "*EWSR1-CREB11*," and "*EWSR1-CREM*." References from relevant publications were crossed referenced and yielded further articles. Excluded were cases where the translocation was inconsistently reported in limited numbers of cases of a tumor type and were thus less likely to be defining. The articles reviewed were limited to the English language. Data collected included tumor name, *EWSR1* translocation partner, histology, Immunohistochemical (IHC) markers, presence of clear cells, patient demographics and anatomic location were documented, when available.

Results

The PubMed search yielded 162 articles and through title and abstract review, 103 articles were included. A total of 17 neoplasms including CCOC and CCC were described in the 103 articles.

In addition to CCOC and CCC, 6 tumors were found to consistently bear the translocations *EWSR1-ATF1*, *-CREB1*, *-CREM*: angiomatoid fIbrous histiocytoma (AFH), clear cell sarcoma (CCS), clear cell sarcoma-like tumor of the gastrointestinal tract (CCSLTGT), intracranial myxoid mesenchymal tumor (IMMT), malignant gastrointestinal neuroectodermal tumor (M-GNET), and primary pulmonary myxoid sarcoma (PPMS). Similarities in gene fusions as well as IHC markers, anatomic location, presence of clear cells and patient demographics are summarized in Table 1.

Tumor	*EWSR1* partner	IHC (+)	IHC (-)	Site	Sex	Age	Clear cells
CCOC	*ATF1* *CREB1* *CREM*	EMA; CK19; AE1/AE3	Desmin; SMA; Vimentin	Mandible; Maxilla	F	40s	Present
AFH	*ATF1* *CREB1* *CREM*	ALK; Desmin; CD68; EMA; CD99	CD34; SMA; S-100	Limb extremities; Head and neck	F	5-25	Sometimes present
CCS	*ATF1* *CREB1* *CREM*	S-100; Vimentin; HMB45; MITF; AE1/AE3; Melan-A; PAS	CK19; EMA; SMA; Desmin; CAM5.2	Whole body	F	30s	Present
CCC	*ATF1*	AE1/AE3; CAM5.2; PAS; EMA; p63	S-100; SMA; PASD; Calponin	Salivary glands	F	40s	Present
M-GNET	*ATF1* *CREB1*	S-100; SOX 10; Vimentin	AE1/AE3; SMA; Melan-A; Desmin	Stomach; Intestines	N/A	40s	Present
CCSLTGT	*ATF1* *CREB1*	S-100; SOX10; Vimentin	HMB-45; Melan-A; Tyrosinase; MITF	Stomach; Intestines	N/A	10-80	Sometimes present
IMMT	*ATF1* *CREB1* *CREM*	Desmin; EMA; Vimentin; ALK	AE1/AE3; GFAP; S-100	Intracranial	N/A	20s	Absent
PPMS	*CREB1*	Vimentin; EMA	SMA; Desmin; ALK	Endobronchial	N/A	40s	Absent

Abbrevations: CCOC: Clear Cell Odontogenic Carcinoma; CCC: Clear Cell Carcinoma; IHC: Immunohistochemical; IMMT: Intracranial Myxoid Mesenchymal Tumor; M-GNET: Malignant Gastrointestinal Neuroectodermal Tumor; CCSLTGT: Clear Cell Sarcoma-Like Tumor of the Gastrointestinal Tract; PPMS: Primary Pulmonary Myxoid Sarcoma; AFH: Angiomatoid FIbrous Histiocytoma

Table 1: Fusion partners, IHC markers, anatomic location, patient demographics and presence of clear cells.

CCOC/CCC and CCS tend to appear in both soft tissue and bone. IMMT and AFH both appear in younger patients, with the median age at diagnosis ranging from the teenage years to the early twenties. CCS, CCOC, CCC, PPMS, CCSLTGT and M-GNET all occur on average later in life around middle age, though cases are reported in a wide range of ages. CCS, CCC, CCOC and AFH all have a slight predilection for females, while no sex predilection was noted for M-GNET, CCSLTGT, IMMT or PPMS (Table 1). Immunohistochemically, there was an appreciable amount of variation among all the listed tumors.

Tumors that inconsistently harbored *EWSR1-CREB1* fusions were angiosarcoma, atypical central neurocytoma, malignant epithelioid tumor of the peritoneal cavity, malignant mesothelioma, neuroendocrine neoplasm, primary intracranial neoplasm, pulmonary mesenchymal tumor, small blue round cell tumor and soft tissue myoepithelial tumor. No further data regarding these tumors was collected.

EWSR1 translocations have been reported in some cases of other tumors but the significance of these findings is unclear.

Discussion

Initial tumor classifications based on histological description have been expanded to include immunohistochemical patterns and, more recently, molecular features detected by a variety of techniques. The first description of a tumor attributed to a chromosomal translocation was in a 1956 paper by Albert Levan characterizing mouse tumors [17,18]. This work was verified and expanded on later in the 1950s and '60s by Levan and T.S. Hauschka in Fox Chase, Pennsylvania as well as Peter Nowell and David Hungerford in their work characterizing the Philadelphia chromosome [19,20]. The following decades proved to be fruitful in the search for and characterization of translocated genes in instances of tumor formation18. As genetic descriptions are further developed, tumor classification and nomenclature may change. As tumors become defined and redefined by their genetics, clinical treatments, too, may also be based on genetic translocations and fusion partners, especially within the context of targeted therapies.

Advances in pharmacogenomics have led to specific treatments based on individual genetic information such as in the case of genetically defined breast cancers [21,22]. For example, BRCA1 and BRCA2 are known as regulators of DNA repair, transcription, and cell cycle progression in the context of DNA damage. In addition to the high risk of developing breast cancer for individuals with germline mutations, BRCA1 and BRCA2 are also linked to increased responses to PARP inhibitors. Additionally, in concert with genetic changes found in these tumor cells, a variety of mutations in the genes that are involved in drug metabolism can affect therapeutic responses[21].

Also of interest is that cases exist wherein different tumors with the same mutations respond differently to the same drug therapy. For example, targeting of the *BRAF (V600E)* oncoprotein with the small-molecule drug PLX4032 (vemurafenib) is a highly effective therapy for treatment of melanoma. However, patients with colon cancer harboring the very same *BRAF (V600E)* mutation have relatively poor prognosis and demonstrate a lackluster response to this drug, underscoring the imperative of tumor environment and cellular lineage origination [23]. Furthermore, genomic studies have demonstrated that mutations in similar genes, such as *ARID1A, PIK3CA* and *KRAS*, are seen in different conditions of varying severity-ovarian clear cell carcinoma and benign endometriosis [24]. Interestingly, these mutations are postulated to be in same cell of origin [25] thus underscoring the importance of tumor environment on treatment efficacy. Therefore, a clearer understanding of what defines tumor behavior is crucial in the delivery of appropriate and effective care. Response of tumors to treatment may be based on the cell lineage from which the malignant cells arise, the environment in which the tumor grows and the genetic alterations (i.e., translocations) present.

In the cases of CCOC and CCC, two rare and intriguing tumors, the presence of an *EWSR1* translocation with a fusion partner in the *CREB* family of proteins seems to be characteristic [6,9-15]. This tumor, which was thought to be benign and locally aggressive, was redefined by the World Health Organization in 2005 as a carcinoma with metastatic potential. It is a malignant neoplasm that may sit dormant for many years and appears to be low grade [16]. Of interest is the fact that some benign tumors of the head and neck can be locally recurrent, aggressive, and destructive (e.g., ameloblastoma and odontogenic keratocysts) [26,27], while some malignancies such as low-grade sarcomas, CCOC, and CCC behave less aggressively than expected of a malignant tumor.

EWSR1 translocations are common among many kinds of mesenchymal tumors and their presence helps to classify tumors such as the Ewing sarcoma family of tumors. These tumors are cytogenetically defined by rearrangements of the *EWSR1* gene at 22q12 with *FLI1* at 11q24 or *ERG* at 22q12 in 85% and 5%-10% of cases, respectively. Less than 1% of Ewing's tumors have other fusion partners such as *ETV1* at 7q22, *E1AF* at 17q12 or *FEV* at 2q33 [28-31].

Animal models can provide more tissue for study which is of great significance in these rare tumors. They will also allow the study of specific tumors, the cell lineage, genetics, and anatomic location. Understanding these interactions will aid in selecting appropriate treatments since tumor responses are generally multifactorial.

Mouse models with high disease penetrance can mitigate the challenge associated with rare disease therapy development. Straessler, et al. and Komura, et al. separately developed mouse models of CCS [32,33]. In the former model, Cre floxes a loxP-flanked STOP signal, thereby allowing *EWSR1-ATF1* expression; in the latter, the *EWSR1-ATF1* fusion mutation is expressed under control of the doxycycline-inducible Tet-On system. Both models demonstrated that induction of *EWSR1-ATF1* expression in multipotent mesenchymal cells within connective tissues results in malignant transformation into CCS. Studies to further define how similar CCS and CCOC are may be useful. These models may be significant in the investigation of other tumors with *EWSR1-CREB1* fusions such as CCOC and CCC. Future studies will be required to examine how the *EWSR1-ATF1* mutation modifies the fate of cells in the region of the head and neck. Furthermore, studies are required to determine exactly which cells are susceptible to undergoing pathologic degeneration to COCC, and whether specific environment/niche characteristics contribute to this transition in the setting of the *EWSR1-ATF1* mutation.

Ongoing study of these seven tumors is currently underway based on immunohistochemistry, cell lineage, environment, and genetic translocations, to further elucidate similarities and differences to CCOC.

Conclusion

EWSR1-CREB1 fusions are commonly found in 6 tumor types in addition to clear cell odontogenic carcinoma (CCOC) and lear cell carcinoma (CCC) of the salivary gland. The high frequency of *EWSR1-CREB1* fusions suggests that these tumors may share similar oncogenic mechanisms and therefore may be useful as surrogates for the characterization of our tumors of interest: clear cell odontogenic carcinomaand clear cell carcinoma of the salivary gland.

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