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Review Article

Nuclear Protein in Testis (NUT) Midline Carcinoma: A Rare Aggressive Solid Tumor

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Abstract

NUT midline carcinoma (NMC) is a rare and aggressive solid tumor characterized by chromosome rearrangements on 15q14 of the NUT gene, creating the NUT fusion oncogenes which lead to blockage of squamous cellular differentiation and rapid growth of tumor cells. The BRD-NUT oncoprotein is considered a major pathogenetic driver of cellular transformation. The median survival time of NMC patients is only 6.7 months despite of surgical resection or chemoradiotherapy, and most patients with NMC have been diagnosed with locally advanced or metastatic diseases. Given the rarity and unfavourable outcomes of NMC, it is essential to increase knowledge and awareness of this malignancy in clinicians and pathologists. In this article, we reviewed the previous literatures and concluded the clinicopathological features, molecular mechanism of NUT midline carcinoma. Besides, we provide with accurate diagnostic methods and explore therapeutic opportunities for this extremely rare and aggressive cancer type, and two promising target agents including bromodomain inhibitors (BETi) and histone deacetylase inhibitors (HDACi) are discussed.

Keywords: NUT Midline Carcinoma, Chromosome rearrangement, BRD4-NUT fusion, Clinicopathological features, Molecular mechanism, Diagnosis, Treatment, Prognosis

Background

Nuclear protein in testis midline carcinoma (NMC) is a rare, poorly differentiated and highly aggressive carcinoma that characterized by chromosomal rearrangements of the gene NUT, at 15q14. The bromodomain protein family member 4 (BRD4) gene on 19q13 is the most common translocation partner forming a fusion oncogene, BRD4–NUT [1]. NMC was first discovered by Kubonishi in 1991 and then it was described on a 12 years old girl through chromosome karyotype analysis in 1999 by Dr. Jonathan Fletcher [2]. Finally, the BRD4-NUT fusion oncogene mechanism was first identified by French in 2003 [3]. Hence, the current name of this cancer is NUT midline carcinoma, or NMC, reflecting that it is defined by chromosomal rearrangement of NUT. Since 2010, a centralized international tumor registry, the NMC Registry (www.nmcregistry.org) was established to collect patients demographic and outcome data. It has been a major source of clinical information and awareness for this disease, and by February 2016, a total of 127 cases had been registered, including 40 cases of head and neck as the primary tumor location and two cases involving the parotid gland [4]. Until now, only two large series, one including 54 patients [5], the other 48 patients [6] have been published. And because of the rarity and unfavorable outcomes of this malignancy, it is important for clinicians to learn more about it.

Clinical Presentation

NMC was originally considered as a disease of children and young adults, but recent publications have shown that NMC can equally affect individuals at any age (0-78 years, median 16) and both males and females are affected equally [5]. Indeed, the designation ‘‘midline’’ is applied to NMC because NMC usually arises within midline structures in the head and neck or in the thorax (including nasal cavity, sinus, nasopharynx, epiglottis, larynx, mediastinum, lung) [7,8]. Besides, other sites including bladders, iliac bone and kidney have been reported as well [2,9]. Our review of the literature revealed that, there are no known associations with exposures to environmental toxins or infectious agents, smoking, or oncogenic viruses such as human papillomavirus or EpsteineBarr virus. In the majority of cases, patients with NMC present with advanced disease. Most common symptoms are local mass and pain caused by tumor cells infiltrating,

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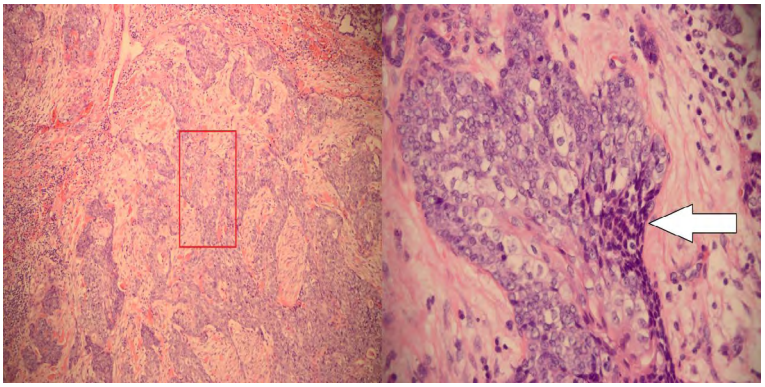

Figure 1: Histomorphology of pulmonary NUT midline carcinomas: abrupt squamous differentiation is difined as largely poorly differentiated the tumor cells with island of well-differentiated squamous epithelium (as shown in squared rectangle) (H&E stain, ×40) (A). Higher magnification shows poorly differentiated tumor cells that most of these were discohesive, monotonous, atypism, scanty cytoplasm tumor cells and irregular ovoid hyperchromatic nuclei (as shown by arrow). (H&E stain, ×400) (B).

oppression and diffusion. Some patients remain asymptomatic for extended periods of time, and diagnosis is made when tumor burden is signifcant.

Pathologic Features

Histologically, the tumor cells were largely poorly differentiated and most pathology specimens typically show abrupt squamous differentiation (Figure 1). Nests and sheets of primitive cells with high nuclear/cytoplasmic ratios imparting a blue, low-power appearance. The tumor nuclei were round to oval with even chromatin, a single delicate nucleolus, and little variability in size and shape [9].

NMC is morphologically categorized as a variant of squamous cell carcinoma and we consider it a epithelial tumor currently. NMCs are mostly immunoreactive with antibodies to p63, cytokeratin, CK20, CD34 [10]. Tumors have reportedly been non-reactive with antibodies to desmin, myoglobin, smooth muscle actin, muscle actin, chromogranin, synaptophysin, leukocyte common antigen, placental alkaline phosphatase, S100 protein, alpha-fetoprotein, neuron specific enolase, CD57, CD99, and HMB45 [10].

Recently, a highly sensitive and specific monoclonal immunohistochemical test for NUT (C52 monoclonal antibody) was introduced, which greatly simplified the diagnosis. Positivity was defined as strong, speckled nuclear staining in greater than 50% of nuclei (Figure 2).

Molecular Findings

NMC is genetically defined by translocations involving NUT gene on chromosome 15q14 [1]. In approximately 70% of cases, the NUT gene is fused to the BRD4 gene on chromosome 19p13.1 by translocation t (15;19)(q14;p13.1), resulting in the BRD4-NUT fusion [4], and in the remaining cases, the NUT gene either fused to the bromodomain protein family member 3 (BRD3) gene on chromosome 9 by translocation t(9;15) (q34.2;q14) forming the BRD3-NUT fusion [11], or the (nuclear receptor binding SET domain 3) NSD3 gene on chromosome 8 by translocation t(8;15)(p11.23;q14) forming the NSD3-NUT fusion, or fused to other uncharacterized genes forming NUT-variant fusion [12]. Uncommonly, a unique cytogenetic abnormality of three-way translocations have been reported in NMC, including t(4;15;19)

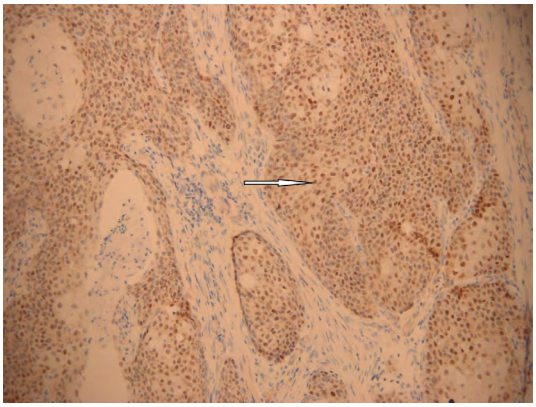

Figure 2: Immunohistochemical stain for NUT: Immunohistochemical analysis was completed by NUT (C52B1) Rabbit mAb(® 2014 Cell Signaling Technology, Inc.) which was able to detect NUT protein. Brown represents positive staining. Strong nuclear reactivity of NUT in the tumor cells is indicated by the white arrow, and positivity was defined as strong nuclear staining in greater than 50% of nuclei.

[13], t(9;15;19) [14], t(11;15;19) [15] and t(2;9;15) [11]. The characterisation of NUT fusions can be detected by the techniques of FISH, or reverse-transcriptase polymerase chain reaction (RT-PCR). Although it is not required for diagnosis, determination of the specific fusion oncogene may influence treatment decisions in the future.

In 2008, Dr CA French provided the first evidence that BRD-NUT fusion proteins play an critical role in NMCs. The research investigated the effects of siRNA-induced BRD3-NUT and BRD4–NUT withdrawal, silencing of these proteins in NMC cell lines resulted in squamous differentiation and tumor cell growth arrest, which suggested that BRD-NUT fusion proteins contribute to carcinogenesis by associating with chromatin and interfering with epithelial differentiation [11]. In 2015, an experimental study found the oncogenic BRD4-NUT chromatin regulator drives aberrant transcription within large topological domains, they found that BRD4-NUT forms distinctive nuclear foci in tumors, which correlate with ~100 unprecedented, hyperacetylated expanses of chromatin that reach up to 2 Mb in size, and these "megadomains" appear to be the result of aberrant, feed-forward loops of acetylation and binding of acetylated histones that drive

transcription of underlying DNA in NMC patient cells induced to express BRD4-NUT. Besides, they found the cMYC and TP63 regions are targeted in all NMCs and play functional roles in tumor growth [16].

As we know, abnormal microRNAs (miRNA) expression promotes tumour formation by modulating the functional expression of critical genes. In 2017, one study revealed a set of 48 miRNA might be dysregulated to target the critical genes other than the parent genes (BRD4 and NUT), causing the cancer, and they assumed that amplification in the expression level of these miRNAs can be used for NMC diagnosis and prognosis [17].

However, the detailed molecular mechanisms underlying the NMC are still not clear and new findings are urgently required to complement the current findings. In addition, a better understanding of molecular mechanisms can help exploring novel therapy for NMC patients.

Imaging

CT with intravenous contrast is standard for initial staging, also, it is a choice for surveillance. NMC typically appearing as a hypoattenuating heterogeneously enhancing infiltrative mass with poorly defined margins and internal necrosis, with local invasion [18]. Enhanced MRI should be performed if the tumor is located in head and neck because of the complex anatomical structure. The signal intensity on T2-weighted MRIs is that of a cellular neoplasm, but imaging characteristics are otherwise indistinguishable from other high-grade neoplasms such as lymphoma or sarcoma [18]. FDG-PET/CT is the preferred modality for guiding biopsy to viable tissue, staging and assessment of metastatic disease, evaluation of disease treatment response, and restaging with assessment of disease extent and severity over time [18].

Diagnosis

NMC is difficult to diagnose for oncologists and pathologists because of its rarity and non-specific histology. There are only a few individual case reports and small case series, besides, no risk factors or specific symptoms, signs or characteristic imaging features of NMC were found. The diagnosis of NMC is made definitively by demonstration of NUT rearrangement by Fluorescence In Situ Hybridisation (FISH) or by demonstration of a BRD-NUT fusion transcript by RT-PCR. FISH is preferred because it will detect all NMCs, including all NUT variants (Figure 3), whereas RT-PCR can currently only detect BRD3-NUT or BRD4-NUT tumours. Beginning in 2009, the diagnosis of NMC is made by immunohistochemical demonstration of nuclear reactivity for NUT using a monoclonal antibody available from Cell Signaling Technologies. Therefore FISH, RT-PCR, or cytogenetic analysis is no longer required for this diagnosis as the specificity of the NUT antibody is 100 % and the sensitivity is excellent at 87 % [19]. The only tumors which could also display nuclear NUT reactivity were germ cell tumors. However, the staining is very focal (<5%), faint, and lacked the speckled pattern. While positivity in NMC is defined as strong, speckled nuclear staining in greater than 50% of nuclei [19]. The biggest challenge is not the diagnosis of NMC, but to determine when to perform the test. In our opinion, IHC staining for NUT should be considered when the histopathological features are met, besides, FISH, RT-PCR, or cytogenetic analysis is required if necessary. The frequency of diagnosis of NMC

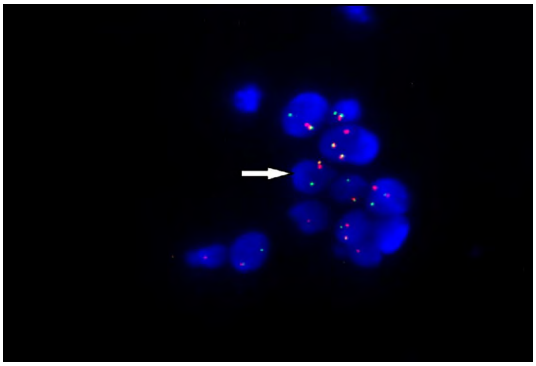

Figure 3: Fluorescence in situ hybridization testing: The Dual Color Break Apart Probe is a mixture of two direct labeled probes hybridizing to the 15q14 band. The green fluorochrome direct labeled probe hybridizes proximal and the orange fluorochrome direct labeled probe hybridizes distal to the NUTM1 gene. In an interphase nucleus lacking a translocation involving the 15q14 band, two orange/green fusion signals are expected representing two normal (non-rearranged) 15q14 loci. A signal pattern consisting of one orange/green fusion signal, one orange signal, and a separate green signal indicates one normal 15q14 locus and one 15q14 locus affected by a translocation (as indicated by the white arrow).

appears to be increasing, particularly since 2012, this may be an effect of the improved diagnostic availability of a simple, highly sensitive and specific immunohistochemical stain for the NUT gene product.

The differential diagnosis for NMCs are undifferentiated carcinomas and poorly differentiated carcinomas. It includes pediatric small blue cell tumors (primitive neuroectodermal tumor, rhabdomyosarcoma, desmoplastic small round cell tumor, etc.), melanoma, olfactory neuroblastoma, high-grade hematologic malignancies, endocrine carcinomas, sinonasal undifferentiated carcinomas and EBV-driven non-keratinizing squamous cell carcinomas which occur most frequently in the nasopharynx [10].

Treatment and Prognosis

To date, no specific treatment exists for NUT carcinoma. Based on the clinical data analysis reported, the majority of the patients received intensive initial multimodality therapy, which consisted of various combinations of surgery, chemotherapy, and radiotherapy. Surgical resection is the corner stone of therapy, radiotherapy and chemotherapy are considered as adjuvant treatment. As the previous literature analysis reported, different intensive chemotherapy regimens have been used, and these chemotherapy regimens include platinum, taxanes, anthracyclines and non-platinum alkylating agents [5,6,14]. The best responses were observed for anthracycline based, alkylating agent based, and cisplatin based chemotherapy [20]. Other second-line or subsequent conventional chemotherapy combinations were not effective. Responses to chemotherapy with anthracyclines and ifosfamide have also been reported in the literature, but with limited efficacy [20].

The largest retrospective analysis of NMC with 54 patients had been performed [5]. In the study, surgical extent was classified as gross total resection or less than gross total resection, and chemotherapy was categorized into regimens containing either cisplatin and carboplatin, or regimens containing anthracyclines and non-platinum alkylating agents. Many patients received

combination therapy. They found the extent of surgical resection and initial radiotherapy were independently predictive of progression-free survival (PFS) and overall survival (OS). Patients who received less than a gross total surgical resection or no surgical resection had a five-fold greater risk of progression and eight-fold greater risk of death compared with patients who underwent gross total surgical resection. Patients who did not receive initial radiotherapy had a 2.8 times greater risk of progression and 2.2 times greater risk of death compared with patients who received initial radiotherapy. The most striking finding of this study is the extremely poor prognosis of patients diagnosed with NMC, who have an 6.7 month median survival and a greater than 80% likelihood of death within the first year after diagnosis for adult patients.

Another retrospective review of head and neck NMC (HNNMC) in the International NUT Midline Carcinoma Registry was performed [6], 48 HNNMC patients were enrolled, OS and PFS according to patient characteristics and treatment were analyzed. The initial treatment was initial surgery with or without adjuvant chemoradiation or adjuvant radiation, initial radiation with or without chemotherapy, initial chemotherapy with or without surgery or radiation. The result showed that the median PFS was 6.6 months (range, 4.7-8.4 months), the median OS was 9.7 months (range, 6.6-15.6 months), the 2-year PFS rate was 26%, the 2-year OS rate was 30%. They suggested initial surgery with or without postoperative chemoradiation or radiation and complete resection with negative margins were significant predictors of improved OS.

In the most recent retrospective multicenter study [20], a series of 12 NMC patients had a median age of 18.1 years were enrolled, the median overall survival was 4.7 months. This study confirms the poor chemosensitivity of NUT carcinoma, with a very transient response rate to chemotherapy of approximately 36%, rapidly followed by tumor progression.

By reviewing the relative literatures, we conclude that there was no statistically significant difference in PFS or OS by age, sex, tumor location, size, histology, presence of neck lymph node involvement, or BRD4-NUT translocation.

Targeted Agents

The rapid growth and aggressivity of NMC indicate that surgical treatment is not enough, and it appears that the efficacy of most chemotherapeutic regimens or radiation therapy are limited. Some promising therapies, bromodomain inhibitors (BETi) and histone deacetylase inhibitors (HDACi), are currently under study and perhaps these targeted agents will provide an effective treatment in the future.

BET proteins is a family of proteins includes BRD2, BRD3, BRD4, and BRDT. The role of BRD3 and BRD4 are known as mutant oncoprotein fused to the p300-recruiting NUT protein in NMC. BETi are acetyl-histone mimetics which bind BET bromodomains, competitively inhibiting its engagement with chromatin [21]. The antineoplastic effects of BETi were first demonstrated in NMC and have since been shown to be effective at inhibiting the growth of many different cancers [21]. So far, at least 11 clinical trials using BETi in cancer have been initiated, and 3 clinical trials using in NMC, including NCT01987362 (drug TEN-010), NCT02259114 (drug OTX015/MK-8628), NCT01587703 (drug GSK525762) [22]. The results of those trials have not published, however, the preliminary findings have been encouraging. After a novel oral BETi named OTX015/MK-8628 with preclinical impressive and rapid antitumor activity in NMC in a study, two patients achieved notably longer overall survivals (19 and 18 months, respectively) than the median survival of 6.7 months reported in the largest retrospective series of patients with NMC [5,23], and the main side effects were mild to moderate gastrointestinal toxicity and fatigue, and reversible grade 3 thrombocytopenia. A major concern for use of BET inhibitors as targeted therapy of cancer is that it is in theory a rather blunt, not precision instrument, thus, whether a therapeutic window exists where drug levels sufficient to inhibit tumor growth are below dose-limiting, toxicity [22]. It is expected that results from ongoing clinical trials will be published soon, these questions will be answered.

The BRD4-NUT oncoprotein can bind and activate histone acetyl-transferase leading to acetylate chromatin and creating a feed-forward mechanism. HDACi artificially increase acetylation, leading to BRD4-NUT function reversal and thus a return to regular cellular progression [24]. There are some findings suggest that dual function HDAC and PI3K inhibitor CUDC-907 is an effective agent targeting MYC and thus may be developed as potential therapy for MYC-dependent cancer cells, such as NUT midline carcinoma cell [25]. There is a clinical trial investigating the use of a recommended Phase 2 dose of a dual PI3 kinase/HDAC inhibitor drug, CUDC-907, that has been shown to have potent activity against cultured NMC cells (information about can be found at www.nmcregistry.org), it is a open label, multi-center study to assess the safety, tolerability and pharmacokinetics of CUDC-907 in subjects with advanced/relapsed solid tumors.

Indeed, preliminary studies using histone deacetylase inhibitors and BET inhibitors have shown promising results both in vitro and in vivo, and clinical trials using these agents are forthcoming. We should encourage patients diagnosed with NMC to consider participating in clinical trials as early as possible.

Conclusion

NUT midline carcinoma is a rare, aggressive and poorly differentiated solid tumor defined by chromosomal rearrangements of the gene NUT. The biggest challenge for NMC is that because it is a newly described, rare disease that cannot easily be distinguished from other cancers, thus, many pathologists and clinicians are unaware of it, and it is vastly underdiagnosed. Therefore, it is necessary to keep a high level of suspicion for a midline lesion malignancy with poorly differentiated or undifferentiated features, especially with focal squamous differentiation. When histopathological features met, sending genetic testing or immunohistochemical staining for the NUT is recommend. To date, no standard treatment exists for NUT carcinoma, surgical resection and radiochemotherapy is obviously not enough in terms of survival. The two promising therapies including BET inhibitors and histone deacetylase inhibitors are currently under study and perhaps they will hopefully provide an effective treatment for NMC patients in the future.

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Declaration of conflict of interest

None

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