Advances in the pathogenesis and treatment of NUT carcinoma: a narrative review

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Abstract: NUT carcinoma (NC) is a rare, highly invasive and fatal tumor and often misdiagnosed. It typically arises from the mediastinum and midline organs and has complicated pathogenesis and poor outcome. Genetically, its pathogenesis is related to a chromosomal rearrangement involving the NUTM1 gene. In most cases, the main oncoprotein is BRD4-NUT with a translocation between NUTM1 and BRD4 genes, but in a few cases, the oncoprotein is BRD3-NUT, or NSD3-NUT. Studies have shown that the histone hyperacetylation and BRD4 hyperphosphorylation may lead to the activation of cancer circuits. Abnormal production of microRNA, inactivation of tumor suppressor genes and abnormal activation of several signaling pathways are proposed as potential mechanisms underlying the pathogenesis of NC. Currently, there is no consensus on its standard treatment for NC. Extent of surgical resection with negative margins, initial radiotherapy and part of chemotherapy regimens may significantly associated with the improvement of progression-free survival (PFS) rate and overall survival (OS) rate. Some bromodomain and extraterminal inhibitors (BETis) have shown encouraging results in the clinical trials on NC, but delayed drug resistance is still an important issue that needs to be resolved. Histone deacetylase inhibitors are also found to possess the potential in the treatment of NC. Herein, we summarize recent advances in the pathogenesis and treatment of NC.

Keywords: NUT carcinoma (NC); pathogenesis; gene rearrangement; therapy; bromodomain and extraterminal inhibitors (BETis)

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Introduction

NUT midline carcinoma (NMC) is a malignant tumor with involvement of testicular nuclear gene rearrangement, which has the tendency to arise from midline anatomical sites, and it was initially described as a mediastinal tumor in 1991 (1) with increasing cases reported in non-midline structure such as renal pelvis, pancreas, parotid gland, bladder, sublingual gland and femur (2-7). WHO redefined NMC as NUT carcinoma (NC) in 2015 (8). Generally, NC progresses rapidly. Bauer et al. investigated the clinical characteristics of 57 patients with NC and found that the median survival time was 6.7 months (9). Pathologically, NC is characterized by variable degrees of squamous differentiation with a predominance of the poorly or undifferentiated component(10,11). The pathogenesis of NC is complex and related to the acquired chromosomal rearrangements involving NUTM1 (NUT Carcinoma Family Member 1) and other causes leading to the differentiation blocking (12). NC has no preference for gender or age and is often misdiagnosed. The survival rate of NC patients has not been improved significantly.
although some strategies have been employed for the treatment of NC, and there is no consensus on the standard treatment for NC currently. Herein, we summarize the advances in the pathogenesis and treatment of NC in recent years. We present the following article in accordance with the Narrative Review reporting checklist (available at http://dx.doi.org/10.21037/tcr-20-1884).

Pathogenesis

Exposure factors

The occurrence of NC has not been reported to be associated with any special exposure factors such as tobacco use (13) and infection with Epstein-Barr virus or human papillomavirus (14,15). Whether the occurrence of NC is related to other tumor-causing viral infections is needed to be further studied.

Multiple gene rearrangement

Malignant tumors may be caused by genetic changes, including the acquisition or loss of chromosomes and chromosomal segments, gene translocation, gene transcing, and gene point mutations, which can lead to the activation of oncogenes or inactivation of tumor suppressor gene (TSG). Shiota et al. found that NUT is expressed in post-meiotic spermatogenic germ cells, and it can recruit p300 and/or CBP and enhance histone H4K5 and H4K8 acetylation, leading to the histone-to-protamine replacement (16). In the rearrangement gene expression of NC, the NUTM1 (NUT) gene usually uses bromodomain-containing protein 4 (BRD4) as a fusion partner, sometimes uses BRD3, and, in a few cases, uses NSD3 (17,18). Alekseyenko et al. found that ZNF532 as a chromatin factor could interact with BRD4-NUT complexes, and they also identified another fusion oncogene, ZNF532-NUT (19). It has been reported abnormal three-way translocations involving t (4;15;19), t(9;15;19), t(11;15;19) and a t(2;9;15) in NC cells (20). A new type of NUT fusion partner MGA has also been reported (21,22), but this pathology is ultimately classified as a sarcoma; MXD4, a novel partner, is reported recently (21,23).

Lee et al. sequenced the whole genome and transcriptome of three NC patients and found that, except for BRD3/4-NUT oncogene rearrangement, no canonical oncogenes or tumor suppressor genes were affected, but it caused NC, a fatal disease (13). French et al. for the first time reported the fusion oncogene, BRD4-NUT in 2003 (24). The expression of NUT fusion oncogenes has great heterogeneity at different ages and tumor locations, which indicates that NC may eventually be divided into clinically related subgroups with different clinical outcomes and therapeutic responses (25). Chau et al. grouped 124 out of 141 NC patients by anatomic location and fusion type, and nonthoracic primary NC group with non-BRD4-NUT fusion had the best outcome (26). Testing for NC rearrangement may be not enough, and the identification of specific fusion partners may be necessary to develop therapeutic strategies.

Histone hyperacetylation and BRD4 hyperphosphorylation

NUT is exclusively expressed in the testis of human and mice, and p300 and/or CBP are the only acetyltransferases present in the NUT interactome. NUT recruits p300 and/or CBP to control histone H3 and H4 acetylation. In NC cells, a chromosomal translocation induces NUT-mediated forced cooperation between p300 and/or CBP and BRD4, forming the hyperacetylated histone chromatin foci (16). This acetylation further affects BRD4-NUT in a synergistic manner, ultimately forming a large number of acetylated chromatin regions covering various topologically relevant domains of the entire genome, called the megadomain (27). The megadomain contains approximately 100 chromatin domains, ranging from 100 to 2,000 kb, across the gene and intergenic regions (28).

BRD4 is a member of the bromodomain and extraterminal (BET) family. The tandem bromodomains of BRD4 (BD1 and BD2) can specifically recognize acetylated histones H3 and H4 on chromatin (29). BRD4 has been implicated in the pathogenesis of a variety of cancers, including hematological malignancies and solid tumors (30-33). It is also the target of genetic translocation between chromosomes 15 and 19, expressed as t(15;19), which leads to the formation of a novel fusion oncogene BRD4-NUT (24). BRD4-NUT can induce the abnormal activation of oncogenes such as SOX2 (34) and c-MYC (35) in the hyperacetylated chromatin in NC cells. Wang et al. found that a unique cellular environment induced BRD4 hyperphosphorylation in HCC2429 NC cells. The hyperphosphorylation of BRD4 induced by BRD4-NUT may contribute to the transactivation of oncogenes, which accounts for NC transformation (36).

SOX2 is a sex-determining region Y-box protein 2 and mainly expressed in the stem cells. SOX2 is an essential transcription factor for the self-renewal and pluripotency...
of stem cells (37). SOX2 mutations induce abnormal self-renewal of stem cells, leading to the abnormal differentiation of stem cells (37,38). **BRD4-NUT** induces abnormally high SOX2 expression in the NC cells to promote the abnormal stem cell-like growth, which is the basis for the highly invasive transformation activity of the t(15;19) translocation (34).

**MYC** up-regulation is a common driving event in some human cancers. **MYC** can maintain the transcriptional programs to promote cell growth and proliferation. Grayson et al. found that **BRD4-NUT** prevented the differentiation of NC by maintaining **MYC** expression (35). The ability of **BRD4-NUT** to diffuse and fill the entire regulatory region of **MYC** gene and other genes may explain the aggressiveness of NC.

**MicroRNA (miRNA) amplification**

miRNAs play a key role in regulating target genes and are involved in the development and progression of cancers. miRNAs refer to a group of non-coding RNAs approximately 21 nucleotides in length that can inhibit the expression of target genes by binding to complementary mRNA. A single miRNA can regulate the expression of some different mRNAs (39,40). Pathak et al. compared the miRNAs that target **BRD4** and **NUT** transcribed regions before and after **BRD4-NUT** gene fusion event. Because of the formation of fusion transcripts, the regions of fusion gene deletion will no longer be the targets for their respective miRNAs. Many such ex-miRNAs will be significantly amplified in cancer cells, resulting in abnormal cell behaviors (41). miR-3140 (a novel tumor suppressor miRNA) directly inhibits the expression of **BRD4** by binding to its coding sequence; it also inhibits the **BRD4-NUT** fusion protein and its downstream target **MYC** in the NC cell lines (42).

**Inactivity of TSG**

**TP53** is a powerful tumor suppressor gene that prevents mutation via DNA repair and inducing apoptosis. There is evidence showing that **BRD4-NUT** promotes acetylation of p53 via p300, resulting in the chelation and inactivation of **BRD4-NUT** (43). It inhibits SOX2 significantly by inhibiting the p300 activity (44); mRNA and protein expressions of c-MYC are also reduced. Alekseyenko et al. found that the expression of **TP53**-associated squamous cell gene **TP63** was regulated by the **BRD4-NUT** megadomain in all tested NCs, which supports a possible mechanism by which **BRD4-NUT** can evade the gatekeeper function of p53 (27).

**Abnormal activation of signaling pathways**

**MYC** maintains the proliferation and undifferentiated state of tumor cells in NC. The blockade of PI3K signaling pathway may inhibit **MYC** activity by suppressing **MYC** gene transcription and protein activity or down-regulating apoptosis of **MYC-dependent** NC cells (44). The activation of RTK, GPCR, and cAMP/PKA signaling pathways are also found to mediate the resistance to **BET inhibitors (BETis)** in NC cells (45).

**Treatment**

**Surgery and radiotherapy**

NC is a highly invasive tumor for which there is no consensus on its standard treatment (6,46-56) (Table 1). It has been reported that extent of surgical resection and initial radiotherapy are the independent predictors of progression-free survival (PFS) rate and overall survival (OS) rate of NC patients (9). A recent study on 48 patients with head and neck NC reported that the only long-term survivors in the study were patients who achieved an early complete remission after initial surgery (57). Radiotherapy as a part of the initial treatment may has a positive impact on the OS rate. However, studies shows that radiotherapy has a positive effect on tumors that originate from the head, neck and lungs, but not on the mediastinal primary tumors (58).

**Chemotherapy**

Currently, several drugs have been used for the chemotherapy of NC, including cisplatin, carboplatin, cyclophosphamide, etoposide, doxorubicin, actinomycin D, vinorelbine, vinblastine, paclitaxel, docetaxel, 5-fluorouracil, S1, bleomycin, vincristine, ifosfamide, gemcitabine and BETis (58). Beesley et al. found that vincristine significantly reduced the tumor burden in NC xenografts and was, therefore, an effective drug for the treatment of NC. However, although vincristine significantly slowed tumor growth in these models, it was not sufficient to prevent tumor recurrence (59). Etoposide and vorinostat have also been used in NC patients. Vincristine, doxorubicin, and
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<td>–</td>
<td>OTX015/MK–8628</td>
<td>Yes</td>
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<td>OTX015/MK–8628</td>
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<td>Arimizu et al., 2018</td>
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<td>Yes</td>
<td>9 months</td>
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<td>Ueki et al., 2014</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>–</td>
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<td>–</td>
<td>Yes</td>
<td>6+ months</td>
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<td>Lorenzo et al., 2017</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<td>HDAC</td>
<td>Yes</td>
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<td>Aizawa et al., 2019</td>
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<td>Oliveira et al., 2019</td>
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<td>–</td>
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<td>–</td>
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<td>Samples et al., 2016</td>
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<td>TEN010</td>
<td>Vorinostat</td>
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<td>D’Souza et al., 2014</td>
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<td>Yes</td>
<td>9 months (in follow-up)</td>
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<td>Ding T et al., 2019</td>
<td>–</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Anlotinib hydrochloride</td>
<td>Yes</td>
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<td>Maher et al., 2015</td>
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<td>Yes</td>
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<td>–</td>
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<td>Vorinostat</td>
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<td>10 months</td>
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<td>Storck et al., 2017</td>
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<td>–</td>
<td>–</td>
<td>Yes</td>
<td>6 years (in follow-up)</td>
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VCR, vincristine; 5-FU, 5-fluorouracil; PTX, paclitaxel; DDP/CBP, cisplatin/kabisa; ADM, amycin; CTX/IFO, cyclophosphamide/isocyclophosphamide; DOC, docetaxel; VP-16, etoposide; GEM, gemcitabine; BETis, bromine-domain proteins inhibitors; HDACi, histone deacetylase inhibitor; OS, overall survival.
flavopiridol (CDK9 inhibitor) show significantly better activity than etoposide and vorinostat; statins and anti-
metabolites exhibit moderate monotherapy efficacy (59).

In two case reports, three pediatric NC patients were treated with a comprehensive protocol for sarcoma
(SSG IX), involving surgery, chemotherapy and focal radiotherapy. These three patients experienced remission
for 6 years, 14 years, and 13 years, respectively (60,61).

**Targeted therapy**

**BET inhibitors**

A BET inhibitor is an acetyl histone mimetic that specifically binds to the BET bromodomain and competitively inhibits its
binding to the chromatin (62). In early studies, BET inhibitors were shown to be effective against murine hematological
malignancies (63). In recent years, many studies have shown that BET inhibitors also effectively inhibit the progression of
non-hematological malignancies (64,65). The anti-tumor effect of BET inhibitors has also been confirmed in NC. NC cells
always have at least one intact BRD4 locus and express normal BRD4 and BRD-NUT oncogenes. A variety of BET inhibitors
are used in clinical trials for the treatment of NC, such as OTX015/MK-8628, GSK525762 and others (such as BAY1238097, GSK2820151, and TEN-010) (62,64,66). Early clinical trials have shown encouraging results (67,68),
especially for hematologic malignancies (34). Pathological examination after tumor biopsy revealed a decreased NUT
expression in areas of differentiation in a NC patient after BET inhibitor treatment. This indicates that NC cells may switch
to a more differentiated squamous cell phenotype after BET inhibitor treatment is initiated. The degree of squamous cell
differentiation after BET inhibitor treatment may be caused by a number of factors, including the duration of treatment and
the site of recurrence (69).

JQ1 is a first-generation BET inhibitor. CDK4/6 inhibitors and JQ1 have been used synergistically in vitro in NC. Endogenous CDK4/6 plays an important role in regulating JQ1 sensitivity, and CDK4/6 inhibitors may exert synergetic effect with JQ1 (45). A study shows that BET inhibitor JQ1 can induce the differentiation and growth arrest in NC cell lines, and also exert anti-tumor effect in xenograft models of NC (70-72).

The small-molecule BET inhibitor birabresib (OTX015/ MK-8628) shows anti-tumor activity in patients with
hematological malignancies. Lewin et al. evaluated the safety and effectiveness of OTX015 in a dose escalation study. Three of 10 patients with NC achieved partial remission.

Pharmacokinetic analysis showed that OTX015 exposure and rapid absorption were associated with a dose ratio
increase, which suggests OTX015 has good safety in the treatment of solid tumors (66,67). BET inhibitors in patients with
BRD3-NUT or NSD3-NUT has similar pharmacodynamic effects; this is the first proof of concept for the clinical activity of bromodomain inhibitors targeting NC (66).

BET inhibitors have reversible adverse effects, including fatigue, headache, thrombocytopenia (66), diarrhea, fatigue, nausea, dyspepsia, and hyperglycemia. The pervasive apoptosis in intestinal cells has also been observed after treatment with BET inhibitors, which may exacerbate the chemotoxic damage or radiation induced injury (73).

**Resistance to BET inhibitors**

BET inhibitors are effective against NC. However, not all NC patients respond to BET inhibitors, and the responders
will eventually develop resistance and relapse (45). Studies have shown that some refractory cancers are resistant to BET inhibitors (74), including NC (42). In some models, resistant cells continue to rely on c-MYC to drive proliferation, but in case of BET inhibitor resistance, resistant cells will switch from BRD4-mediated MYC expression to other pathways, including GLI2 or WNT-β-catenin signaling, to maintain MYC expression (45). NC cells have many potential pathways for maintaining MYC function. ERK and AKT inhibitors can inhibit the expression of downstream signaling pathways of RRAS2 (Ras-associated GTPase). Q1 can significantly reduce the expression of c-Myc and cyclin D1, whereas RRAS2 largely restores the phosphorylation of ERK and increases the expression of c-Myc and cyclin D1. KLF4 (a transcription factor containing a zinc finger structure) mediates Q1 resistance in NC cells, and KLF4 cells are able to maintain MYC and E2F gene expression under Q1 treatment and bypass Q1-induced cell cycle arrest (45). The activation of RTK signaling pathway or GPCR/cAMP/PKA signaling pathway can also mediate BET inhibitor resistance in NC cells. Cell cycle regulators also play an important role in mediating the carcinogenesis of BRD4-
NUT (45). Treatment with PI3KCA inhibitors can induce the sensitivity of drug-resistant cells to JQ1 (75). Binding of a BET inhibitor to a PI3K inhibitor maintains PI3K inhibition and enhances cell killing activity (76). In the drug-resistant cells, the bromodomain of BRD4 is activated and thus it is unaffected by BET inhibitors (77).

**Histone deacetylase inhibitor (HDACi) and small molecules**
Preclinical studies have shown that the upstream modulators, targeting MYC alone, such as histone deacetylase (HDAC) and phosphoinositide 3-kinase (PI3K), can reduce MYC protein expression and inhibit MYC-driven carcinogenesis. CUDC-907 is a small-molecule, double-acting inhibitor of class I and class II HDACs and class I PI3K. It is effective to inhibit the growth and survival of MYC-modified or MYC-dependent cancer cells and may be used as a potential therapy for MYC-dependent cancer. At present, the safety, tolerability, and pharmacokinetic assessments of CUDC-907 have yielded encouraging results in clinical trials (44).

Vorinostat is a histone deacetylase inhibitor (HDACi), and studies have shown that vorinostat can induce tumor cell differentiation and inhibit tumor growth, including NC (78,79). CDK9 is a potential kinase that mediates BRD4 hyperphosphorylation. CDK9 inhibitors can block BRD4 hyperphosphorylation in the NC cells, and a dominant negative inhibitor of BRD4 and CDK9 interaction has been found to abolish BRD4 hyperphosphorylation, oncogene expression, and cell transformation in NC (36). CDK9i and bromodomain inhibition lead to the decrease of MYC protein expression, but only bromodomain inhibition induces cell differentiation (80).

Anlotinib hydrochloride is a novel small-molecule, multi-target tyrosine kinase inhibitor that potently inhibits kinases such as vascular endothelial growth factor receptor, platelet-derived growth factor receptor, fibroblast growth factor receptor, and c-Kit. A patient with NC was relieved after radiotherapy combined with anlotinib hydrochloride. However, more evidence is needed to confirm the therapeutic effect of anlotinib hydrochloride on NC (81).

NC can develop of all ages without gender preference. Only a few patients responded to the treatment, including pediatric cases (52,60,61,66,82). Wang et al. found that children with salivary gland NUT carcinomas represented a distinct subset with male predilection and better overall survival (83). However, due to the small number of cases responding to the treatment, it is not enough to explain the difference in the therapeutic effect between children and adults, and more evidence is needed to confirm this result.

Conclusions

NC is a rare disease and often misdiagnosed due to non-specific pathological and clinical manifestations. It should be considered to avoid misdiagnosis and delayed treatment once poorly differentiated tumor is encountered. The pathogenesis of NC is complicated. At present, there is no consensus on the standard clinical treatment for NC. Extent of surgical resection, initial radiotherapy and chemotherapy may have positive impact on OS rate, although strong conclusion cannot be drawn because of the insufficient number of cases. Comprehensive protocol for sarcoma (SSG IX) and targeted therapy may offer promising options for the treatment of NC.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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