LETTER

# Hypomethylation, endogenous retrovirus expression, and interferon signaling in testicular germ cell tumors

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Methylation of cytosine residues exerts a critical role in silencing gene transcription. Importantly, DNA methylation patterns are often altered in cancer, with many tumors showing site-specific gain of methylation marks in a background of global hypomethylation (1).

In PNAS, Stone et al. (2) show in an ovarian cancer model that treatment with the demethylating agent 5-azacytidine resulted in reexpression of human endogenous retroviruses (HERVs), activation of type I IFN signaling, and increased $CD8^+$ T cells in the tumor microenvironment. These data provide important insights into the role of DNA methylation in suppressing immunogenic pathways and provide additional support for the notion that hypomethylation can resculpt the host antitumor response through derepression of HERV and activation of type I IFN signaling (3, 4).

We hypothesized that such elevated *HERV* expression and type I IFN signaling are also present in tumors that are characterized by constitutive DNA hypomethylation. Seminoma, a type of testicular germ cell tumor (TGCT), provides an opportunity to test this hypothesis. The majority of seminomas are nearly devoid of methylated cytosines (5, 6) and frequently exhibit a brisk lymphocytic infiltrate (7, 8). In contrast, nonseminomatous TGCT, including embryonal carcinoma, teratoma, yolk sac tumors, and choriocarcinoma, show methylation levels in the range of normal tissues and no significant immune cell infiltrate (6, 7). To explore the connection between hypomethylation and the immune infiltrate in TGCTs, we characterized DNA methylation levels in situ and immune infiltrates in a series of TGCTs and observed profound DNA hypomethylation and an increased number of $CD8^+$ lymphocytes in the seminomas compared with nonseminomatous TGCT (Fig. 1 *A*–*C*). This pronounced hypomethylation in seminomas was accompanied by significantly increased expression of HERVs in seminomas compared with other TGCTs, and RNA in situ hybridization revealed that *HERV* expression was restricted to neoplastic cells in seminomas (Fig. 1 *D*–*F*). Further expression analysis using the nanostring Pan-Cancer Immune Profiling Panel in a cohort of TGCTs, including seminoma, embryonal carcinoma, and teratoma, showed IFN-alpha response as the top up-regulated pathway in seminomas. Targeted analysis further revealed increased expression of IFN-alpha (*IFNA1*) in seminomas compared with nonseminomatous TGCTs (Fig. 1 *G* and *H*). Importantly, *IFNA1* expression in seminoma was greatly enriched in neoplastic cells compared with separately microdissected inflammatory cells, suggesting that the IFN response was originating from the neoplastic cells (Fig. 1*I*). Finally, analysis of genes involved in IFN response and viral defense that were shown to be up-regulated by 5-azacytidine (3, 4) were consistently expressed at higher levels in seminomas compared with nonseminomatous TGCT, with their expression being predominantly localized within neoplastic seminoma cells (Fig. 1 *J*–*L*).

These data suggest that constitutive global hypomethylation as observed in seminoma is associated

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with HERV expression, increased type I IFN signaling, and $CD8^+$ T cell infiltrates, providing a plausible mechanism for the dense lymphocytic infiltrates characteristically seen in seminoma.

Amplifying on the findings from Stone et al. (2), we propose that marked DNA hypomethylation, whether tumor-intrinsic or pharmacologically induced, can render tumors more immunogenic and could be used as a potential biomarker for cancer immunotherapy.


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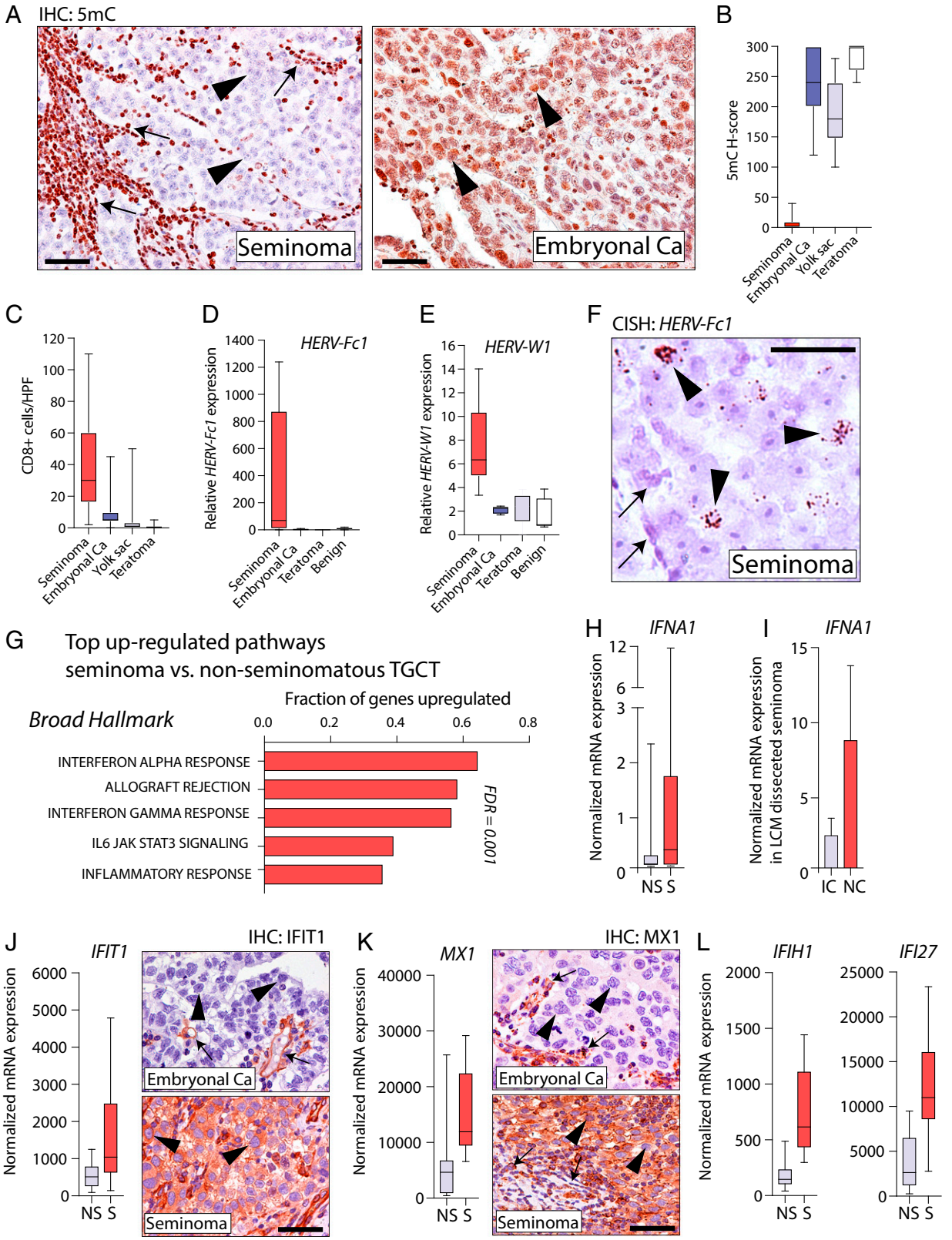


**Fig. 1.** (*A*) Micrographs of TGCTs immunolabeled for 5-methyl-cytosine (5mC) in which seminoma cells (arrowheads) and intratumoral lymphocytes (arrows) are shown. Ca, carcinoma(s). (*B*) Global 5mC levels in 22 seminomas, 14 embryonal Ca, 11 yolk sac tumors, and 9 teratomas. (*C*) $CD8^+$ T cells in TGCTs. HPF, high-power field. (*D* and *E*) Expression of *HERV-Fc1*, *HERV-W1* seminoma ($n = 9$), embryonal Ca ($n = 7$), and teratoma ($n = 3$), as well as benign testis ($n = 3$). (*F*) In situ detection of *HERV-Fc1* is restricted to neoplastic seminoma cells (arrowheads) and not present in adjacent benign cells (arrows). (*G*) Top up-regulated gene sets in seminoma vs. nonseminomatous TGCTs. (*H*) *IFNA1* expression in seminoma (S) and nonseminomatous TGCT (NS). (*I*) *IFNA1* expression in tumor-associated inflammatory cells (IC) and neoplastic cells (NC) isolated by laser capture microdissection (LCM) of seminoma tissues. (*J–L*) Expression of IFN/viral response-related genes. Strong immunoreactivity for IFIT1 and MX1 is present in neoplastic cells (arrowheads) in seminoma, and focal reactivity is present in stromal cells and a subset of tumor-associated lymphocytes (arrows). (Scale bars: 100 μm.)