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## **BK virus–associated urinary bladder carcinoma in transplant recipients: productive or nonproductive polyomavirus infections in tumor cells?**

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### **To the Editor**

We read with great interest the report by Alexiev et al [1] regarding a potential role for polyoma BK virus in urinary bladder urothelial carcinoma tumorigenesis. Several centers, including our own at The University of North Carolina in Chapel Hill, have noticed expression of the SV40-T antigen in some high-grade urothelial and renal tumors arising in kidney transplant recipients. In the future, more studies are needed to evaluate the potential causative role of the polyoma BK virus strain in human oncogenesis. In this context, Alexiev et al have provided some stimulating thoughts for upcoming investigations. Their proposed hypothetical model of tumorigenesis is in part based on the assumption that polyoma BK virus can undergo a replicative cycle in tumor cells. The authors support this model by electron microscopic images interpreted as showing evidence of polyomavirus particles in the carcinoma. However, how solid is the evidence in the current report for a replicative polyomavirus infection in tumor cells?

We take issue with the identification of structures in Figure 3, interpreted to be consistent with polyoma BK virus particles and used to provide evidence of polyomavirus replication. We do not share this view. Alexiev et al [1] could only reprocess formalin-fixed and paraffin-embedded tissue samples for subsequent electron microscopic studies. Such tissue processing for ultrastructural analysis is suboptimal, and preservation artifacts are common, leading to erroneous interpretations. We believe that the “particles” illustrated in Figure 3 do not show virions but rather so-called aggregated nuclear contents [2], also previously described as “nuclear granules” (compare with Figure 23 in [3] and also current Fig. 1,

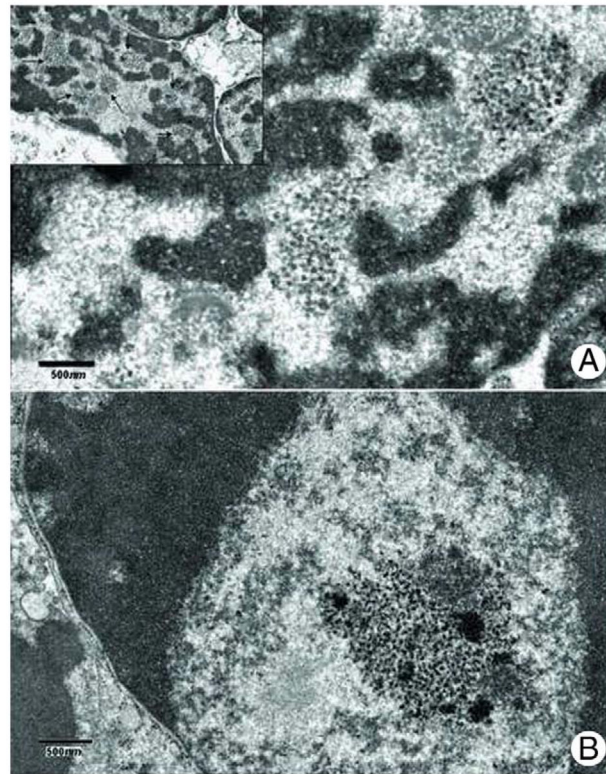
right). Cell nuclei compartmentalize various functions, in particular during phases of increased cell activation and “stress” that appear ultrastructurally as “nuclear bodies” or “nuclear granules.” Such nuclear changes are more prominent in tumor cells and are well recognized [4]. Nuclear granules are known to mimic virions and are diagnostic pitfalls that have been misinterpreted as viruses in the past [2,3]. Thus, based on our assessment and the relatively irregular structure of the illustrated particles in Figure 3, we think Alexiev et al are likely describing “nuclear granules” [3,5,6]. We do not feel that the authors provide convincing ultrastructural evidence of polyomavirus replication.

Moreover, it seems that the molecular studies (all performed on tissue prepared for standard diagnostic workup) do not provide compelling evidence of significant messenger RNA copy numbers for late polyomavirus gene expression, that is, VP1/viral replication. Considering these severe limitations in the study design and interpretation, we ask the following question: do the authors possibly have additional immunohistochemical evidence (using antibodies directed against polyomavirus capsid proteins) or in situ hybridization data to support their notion of productive polyoma BK virus replication in tumor cells?

Based on the currently provided and published data, we do not think that Alexiev et al [1] have convincingly demonstrated polyoma BK virus replication in tumor cells, and the proposed model for BKV oncogenesis (Figure 5 in [1]) remains purely hypothetical. The authors have, however, shown an interesting association of polyoma BK virus with aggressive high-grade urinary bladder urothelial carcinomas that will prompt future targeted studies.

## References

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**Fig. 1.**

A, Originally published as Figure 3 by Alexiev et al [1]. B, High-grade carcinoma from University of North Carolina archives demonstrating nuclear granules. Such structures are often seen in tumor nuclei. The tissue in panel B was fixed in glutaraldehyde before processing and staining for electron microscopy. It is from an intrarenal papillary urothelial carcinoma. Original magnification  $\times 30000$ .