

The deadly bite of STAT3

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The Tasmanian devils' facial tumor disease (DFTD) is a transmissible cancer that spreads by biting and threatens extinction of this marsupial. In this issue of Cancer Cell, Kosack, Wingelhofer, Popa et al. describe how overexpression of ERBB and uncontrolled activation of STAT3 drive DFTD growth and immune evasion.

It is a universally accepted truth that certain types of cancer are caused by viruses and are therefore “contagious”, however a few other types of cancer are transmitted as clonal allografts between genetically unrelated individuals. These transmissible cancers are not caused by viruses or other pathogens, rather they have evolved as “cellular parasites”. Transmissible cancers include the canine transmissible venereal tumor (CTVT), five types of leukemia that infect marine bivalves and two types of tumors that infect Tasmanian devils (DFTDs) (Metzger and Goff, 2016; Pye et al., 2016). Rare as they are, transmissible cancers are remarkable models of cancer evolution, adaptation and immune evasion. CTVT and DFTD are mammalian cancers that, by transmitting between different individuals of the same species, effectively “break the rules of transplantation”, according to which self and non-self cells are recognised by the adaptive immune system and non-self cells are rejected. Key to this histocompatibility barrier is the MHC system. However, it is not clear how these transmissible cancers achieved such an extreme ability to escape immune detection.

In this issue of Cancer Cell, Kosack, Wingelhofer, Popa et al. (Kosack et al., 2019) performed a pharmacological screening to interrogate pathways important for the survival of DFTD cells. They identified several tyrosine kinase inhibitors targeting the epidermal growth factor receptor family (ERBB receptors) that were able to selectively kill cancer cells relative to fibroblasts. They found overexpression and persistent activation of ERBB2 and ERBB3 in DFTD and global transcriptional analysis revealed an upregulation of pathways known to be controlled by ERBB2 and ERBB3. An unbiased proteomic approach on DFTD biopsies detected ERBB-dependent hyperactivation of the transcription factor STAT3 and several of its downstream targets such as the metalloproteinase MMP2, SUMO/ubiquitin E3 ligase TRIM28 and histone deacetylase HDAC5. Conversely, the authors found by pathway enrichment analysis downregulation of chemotaxis, cell adhesion and cytoskeletal remodelling, which are processes known to be negatively regulated by STAT3. These investigations were complemented by integrating DNA methylation analysis of CpG islands in DFTD, which supported the notion that many genes belonging to the ERBB-STAT3 axis found downregulated at the transcriptional level were susceptible to epigenetic control.

STAT3 is critical in cancer development and immunity and is activated by ERBB receptor tyrosine kinase signalling, a pathway often dysregulated in many cancer types. Kosack, Wingelhofer, Popa et al. investigated if pharmacological inhibitors of STAT3 and

downstream targets selectively killed DFTD cells, which was found to be the case. Thus, the ERBB-STAT3 axis was identified as a driver of malignancy for this tumor.

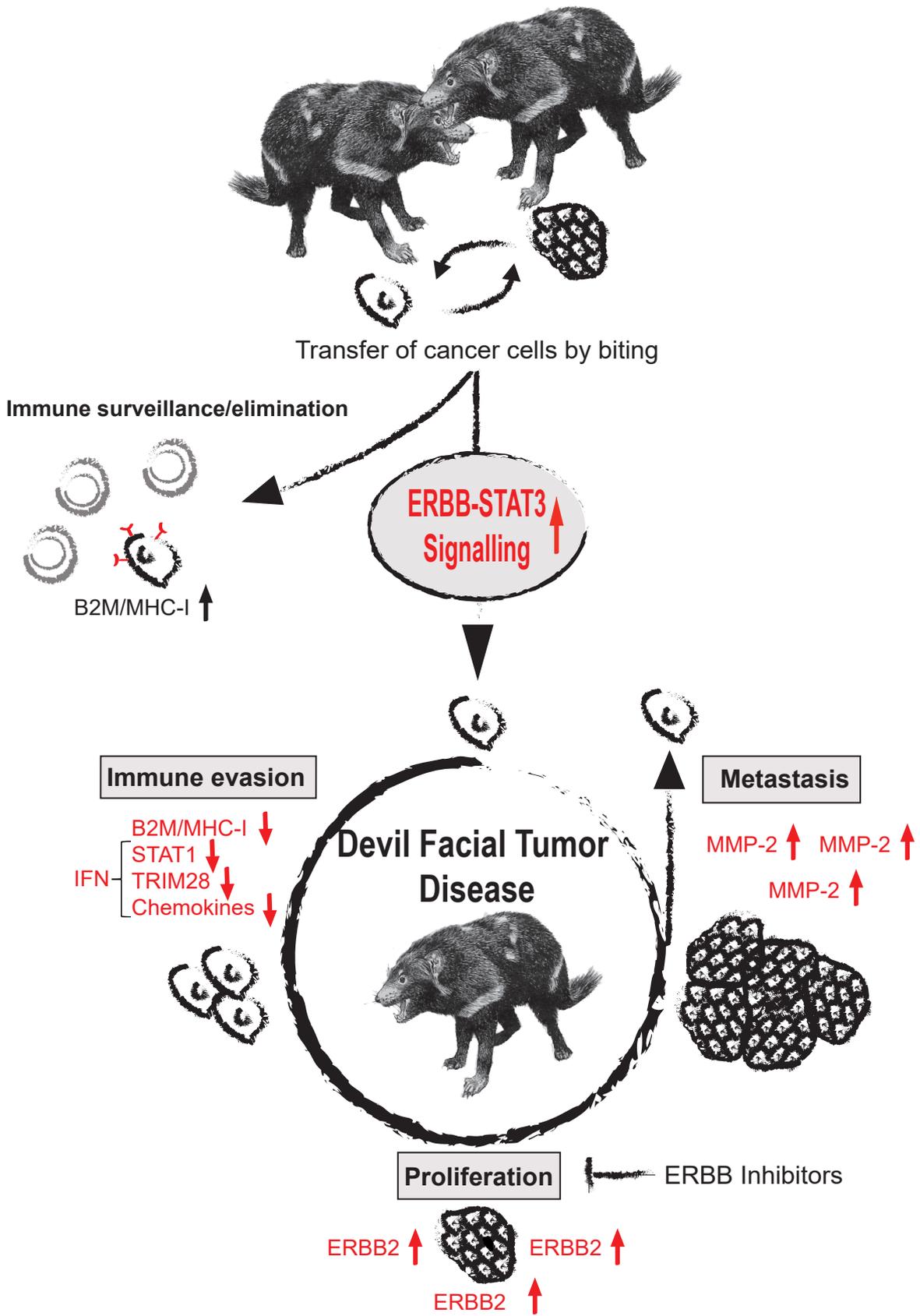
Of the two known types of DFTD (DFTD1 and DFTD2), DFTD1 shows low expression levels of the MHC-I complex and its essential component beta-2-microglobulin (B2M), which appear to be epigenetically repressed. However, repression of these two genes can be reversed upon treatment with interferon gamma (IFN γ) (Siddle et al., 2013). Reversible silencing of MHC-I and B2M is also found in CTVT and is an established mechanism of tumor immune evasion. Kosack, Wingelhofer, Popa et al. then tested if pharmacological inhibition of ERBB could rescue MHC-I and B2M expression. Although this alone was not sufficient, the combination of the ERBB inhibitor with IFN γ produced a synergistic upregulation of both genes, which was mediated by STAT1, a transcription factor activated by IFN γ and repressed by STAT3. Importantly, inhibition of ERBB also impaired growth of DFTD when transplanted into immunocompromised NOD scid gamma (NSG) mice.

The results presented by Kosack, Wingelhofer, Popa et al. indicate that one of the key drivers of DFTD is uncontrolled activation of the ERBB-STAT3 axis, which has two effects: it directly promotes growth and survival of tumor cells and, at the same time, indirectly contributes to tumor immune evasion by repressing MHC-I, B2M and the IFN response (via STAT3 and TRIM28) (Figure 1). This picture agrees with recent data by Stammnitz et al. (Stammnitz et al., 2018) showing that inhibitors of ERBB2 induce DFTD1 cell death. It also provides a rather elegant mechanistic link between cell transformation and escape from immune surveillance.

There are other important aspects of this work that deserve mentioning. Firstly, the results reinforce recent evidence that epigenetic silencing, including transcription factor interference, of immune-related genes may be key to promote cancer immune evasion and resistance to immune checkpoint therapy. Secondly, it suggests that inflammatory stimuli such as IFN may be required to break tolerance to DFTD even if tumor growth is reduced by the ERBB inhibitor. Indeed, the importance of inflammatory cues in rejection of transmissible cancers has been recently demonstrated in CTVT (Frampton et al., 2018). Lastly, DFTD is similar neuroblastoma (Murchison et al., 2010), an incurable cancer of childhood. It may therefore be possible to exploit DFTD as a model for human neuroblastoma, a not so far-fetched idea given that pharmacological inhibition of ERBB has been reported to selectively kill neuroblastoma cells in vitro and in mouse xenografts (Richards et al., 2010).

It is not known if loss of MHC-I and B2M expression is sufficient or even necessary to confer transmissibility to DFTD and CTVT, although tumor transplantation studies in mice indicate that this may not be the case because loss of MHC-I expression triggers rejection mediated by natural killer (NK) cells. Furthermore, DFTD2, discovered more recently than DFTD1, expresses the MHC-I complex on the tumor cell surface yet seems able to transmit between individuals (Caldwell et al., 2018). Recent studies in humans showed that loss of MHC-I heterozygosity conferred an evolutionary advantage to subclonal populations within certain types of cancer (McGranahan et al., 2017), which were also more resistant to immune-checkpoint therapy. Thus, partial loss of MHC-I and B2M expression might be advantageous for clonal selection of transmissible cancers and their wider spread across populations but may

not always be required for the initial evolution of transmissibility. The right ecological niche with limited MHC-I diversity within a population may in part explain the origin of transmissible cancers. However, it is likely that transmissible cancers have evolved additional immune suppressive and immune evasion mechanisms. Understanding what confers transmissibility to DFTD and CTVT will also illuminate mechanisms of cancer immune evasion in general and suggest strategies to reverse them.



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Figure 1.

Mechanisms driving Devil Facial Tumor Disease growth and immune evasion. Tumor cells are transmitted between Tasmanian devils by biting. Hyperactivation of ERBB-STAT3 signalling downregulates expression of the MHC complex, including B2M, and inhibits the IFN response via repression of STAT1, TRIM28 and chemokine genes. ERBB2 hyperactivation promotes tumor cells proliferation and spread/metastasis via upregulation of metalloproteinase MMP-2. Selective ERBB inhibitors reduce tumour growth.