The three axis of *Fusobacterium nucleatum* role in colorectal cancer

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INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed neoplasm of the gastrointestinal tract and the second cause of cancer death. In the past years, sequencing studies in human tumour tissues and stools specimens, along with functional studies in animal models have recognized the possible role of *F. nucleatum* in colorectal carcinogenesis.

OBJECTIVE The aim of this review is to discuss the association between *F. nucleatum* and CRC and to highlight the possible mechanisms of carcinogenesis of this bacterium.

RESULTS

ORIGIN OF *F. nucleatum* TUMOURAL STRAINS AND ENRICHMENT IN CRC TISSUE

Intratumoural *F. nucleatum* strains may have an oral origin as patients with *F. nucleatum*-positive CRC have oral and tumoural *F. nucleatum* strains that share matching arbitrarily primed PCR strain-typing patters[1].

Significantly elevated levels of *F. nucleatum* are found in CRC tissues. The interaction between Gal-GalNAc host polysaccharide, overexpressed in CRC cells, and Fap2 fusobacterial lectin could explicate *F. nucleatum* enrichment in CRC tissues (Fig. 1)[2].
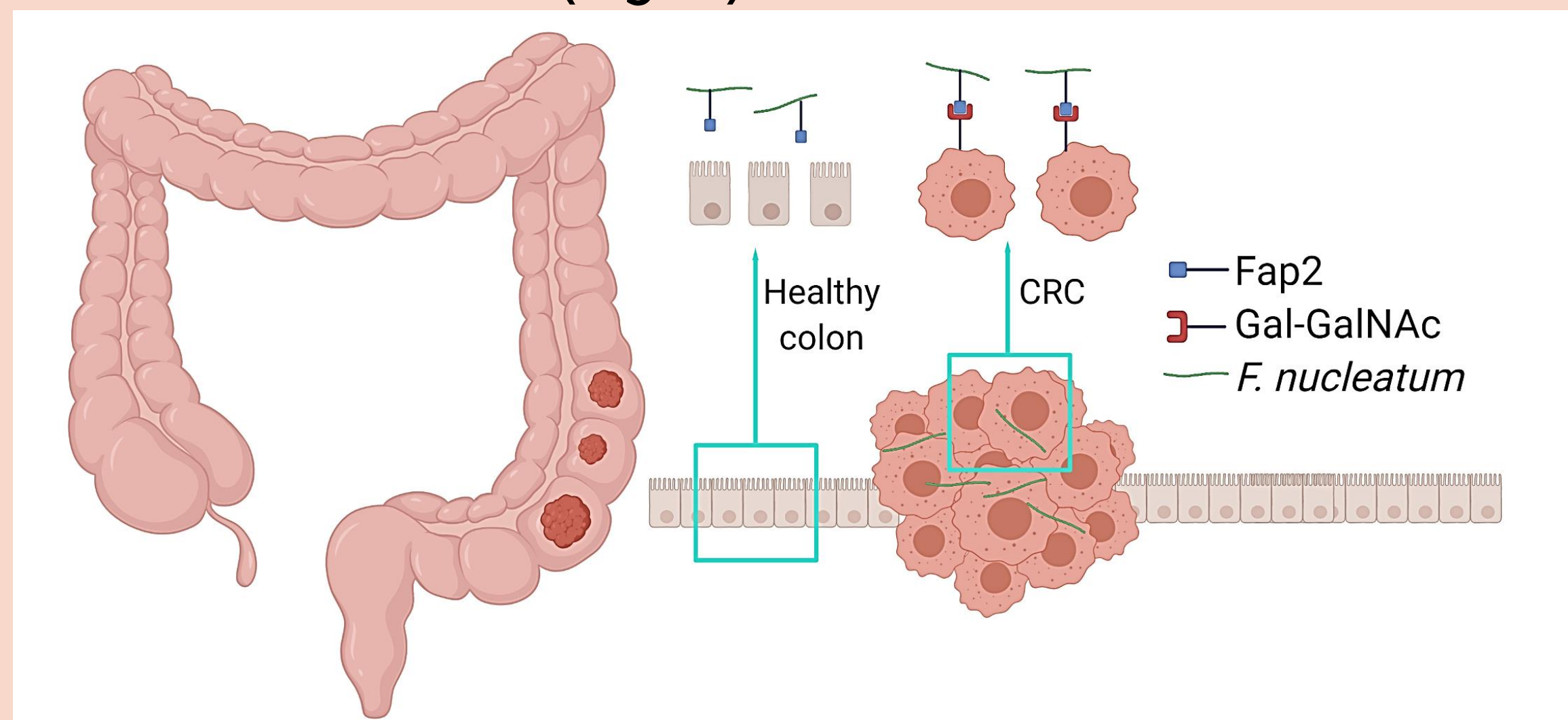

Figure 1. Representation of Fap2-mediated enrichment of *F. nucleatum* in CRC tissue. Healthy colon does not overexpress Gal-GalNAc.

F. nucleatum possible routes to reach CRC tissue to exert its effects:
- **Oral-gastrointestinal route**: mice fed with *F. nucleatum* harbour the bacterium in their large intestine tumours[3].
- **Hematogenous route**: *F. nucleatum*-FadA adhesin colocalizes with vascular endothelial (VE)-cadherin and allows *F. nucleatum* to cross the endothelium through loosened junctions (Fig. 2)[4].
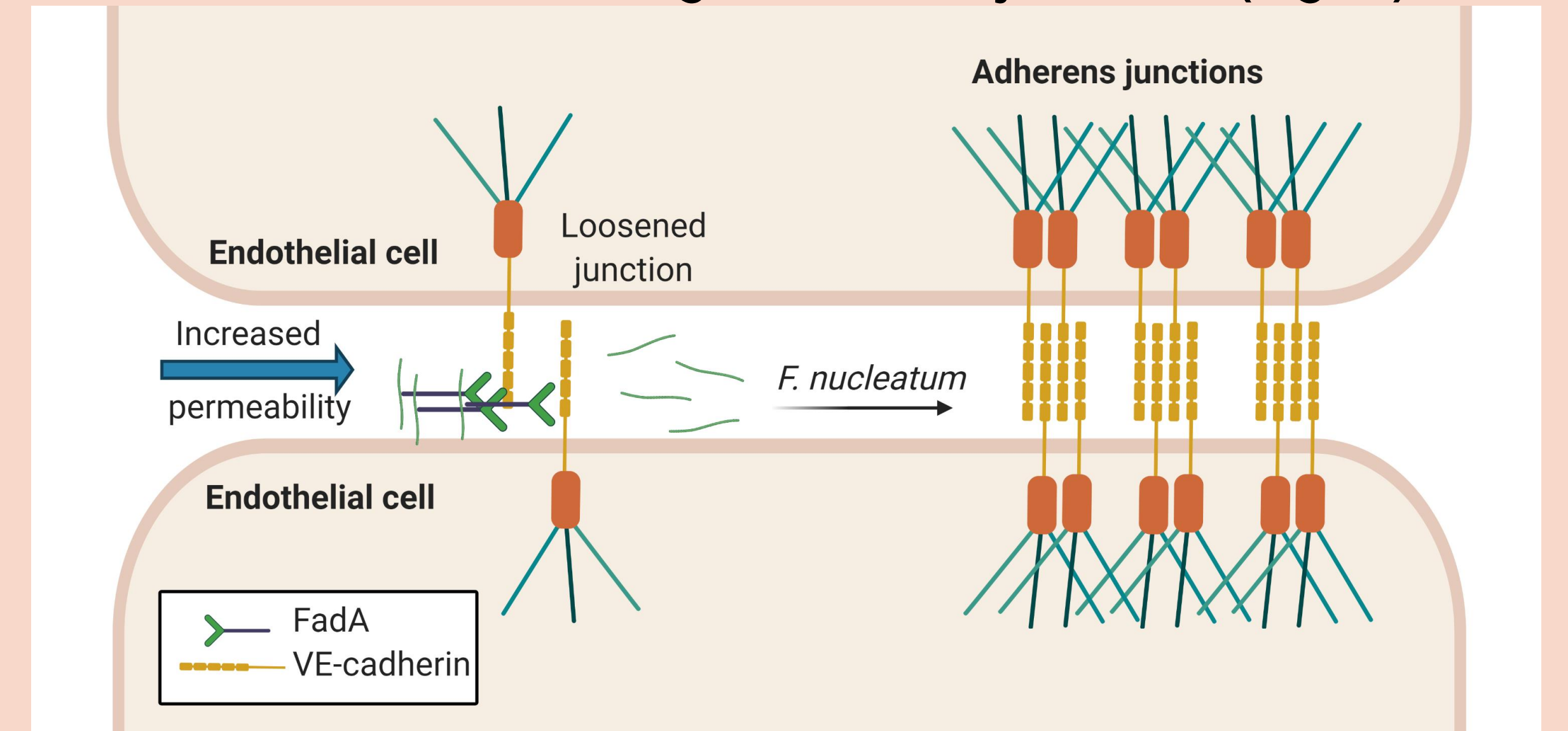

Figure 2. Exemplification of how FadA adhesin binds VE-cadherin and alters endothelial integrity allowing *F. nucleatum* to cross the endothelium.

MECHANISMS OF *F. nucleatum* TO PROMOTE CRC CARCINOGENESIS

1 **F. nucleatum induces tumour cell proliferation**

F. nucleatum-FadA adhesin binds to E-cadherin aided by Annexin A1, which is specifically expressed in proliferating CRC cells. The binding results in a phosphorylation cascade leading to β-catenin accumulation in the cytoplasm and translocation into the nucleus (Fig. 3). Once in the nucleus the β-catenin-regulated transcription (CRT) is activated. Consequently, there is an overexpression of oncogenes such as *CCND1* (Cyclin D1) and *MYC* (c-Myc) genes that induce CRC growth[5, 6].
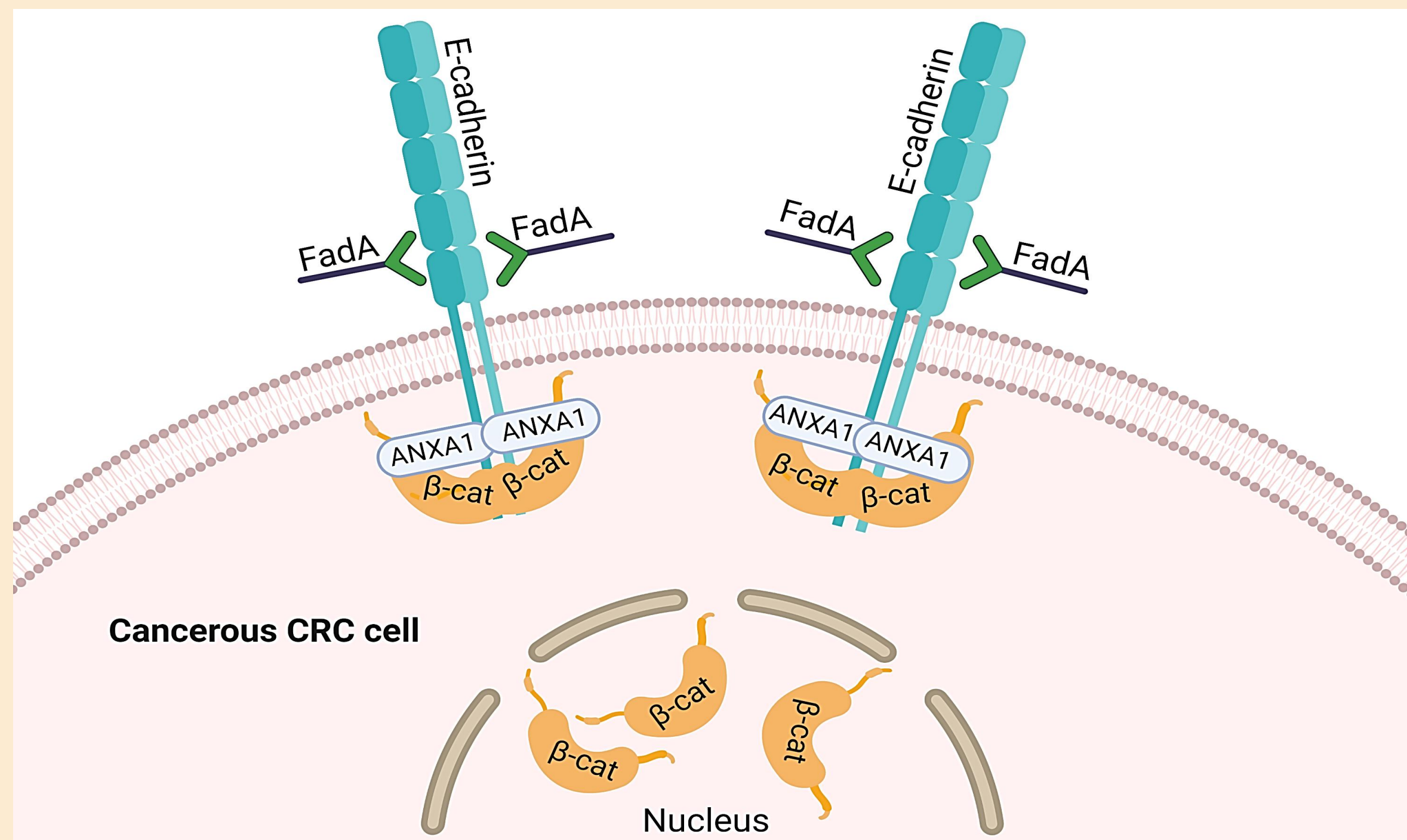

Figure 3. In cancerous cells Annexin A1 level increases, FadA binding enhances, FadA–E-cadherin–Annexin A1–β-catenin complex forms, β-catenin is activated, resulting in acceleration of cancer progression.

2 **F. nucleatum generates proinflammatory tumour environment**

A chronic proinflammatory tumour environment favours tumorigenesis. *F. nucleatum*-associated CRC gene expression signature is enriched for the inflammatory response gene ontology category. This gene expression profile is suggestive of an NF-kappaB-driven proinflammatory response. Indeed, NF-κB signalling pathway is more activated in human CRC samples with high vs low *Fusobacterium* spp. abundance. Experimental evidence suggests that FadA binding to E-cadherin and subsequent internalization via clathrin is necessary for NF-kB signalling pathway activation[3, 5].

- IL1 β
- IL12
- IL6
- IL8
- TNF-α
- TGF-β
- COX-2

3 **F. nucleatum modulates the tumour immune environment**

Selectively recruits tumour infiltrating myeloid cells

Differentiated CD11b+ tumour infiltrating myeloid cells (TIMs) play an important role in promoting tumour progression and angiogenesis [3].
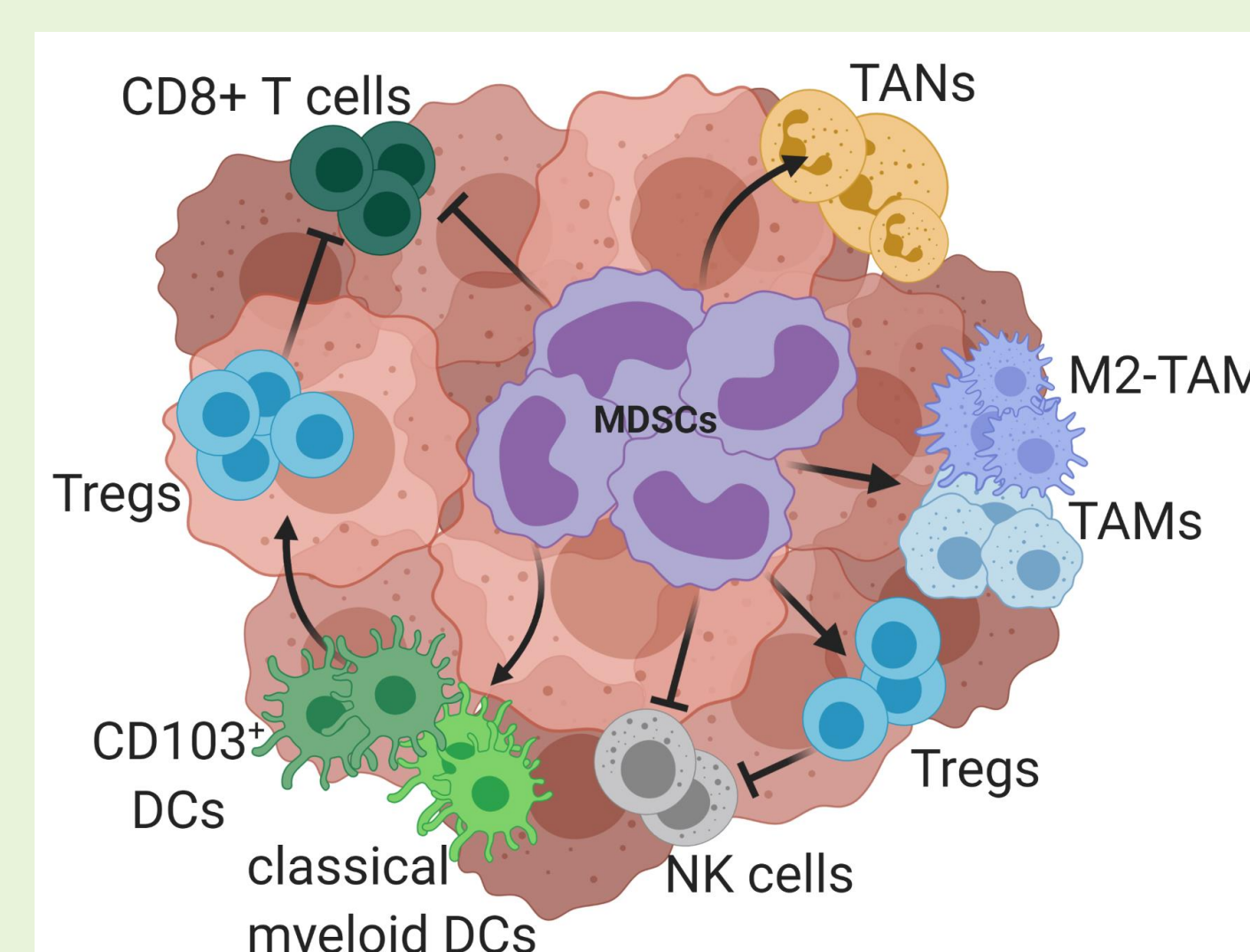

Figure 4. Representative image of TIMs in CRC and their suggested modulation of the immune response to tumour.

Subsets of TIMs are increased in intestinal tumours of $Apc^{Min/+}$ mice fed with *F. nucleatum* (Fig. 4)[3]:

- Myeloid-derived suppressor cells: Monocytic (M)-MDSC and Granulocytic (G)-MDSC
- Tumour-associated macrophages and M2-like TAMs
- Tumour-associated neutrophils (TANs)
- Dendritic cells: classical myeloid and CD103+ regulatory DCs

Suppresses anti-tumour immunity

F. nucleatum directly inhibits the activity of tumour-infiltrating lymphocytes (TILs), i.e. NK, CD4+, CD8+, via two inhibitory immune cell receptors (Fig. 5):

- **Inhibition via TIGIT receptor**: Fap2 protein interacts with TIGIT, thus delivering and inhibitory signal throughout ITIM and ITT domains[7].

- **Inhibition via CEACAM1 receptor**: an unknown *F. nucleatum* ligand, different from Fap2 protein, binds and activates the inhibitory receptor CEACAM1[8].

The additive effect of both *F. nucleatum* ligands helps cancer evade immune cell attack and enables tumour progression.
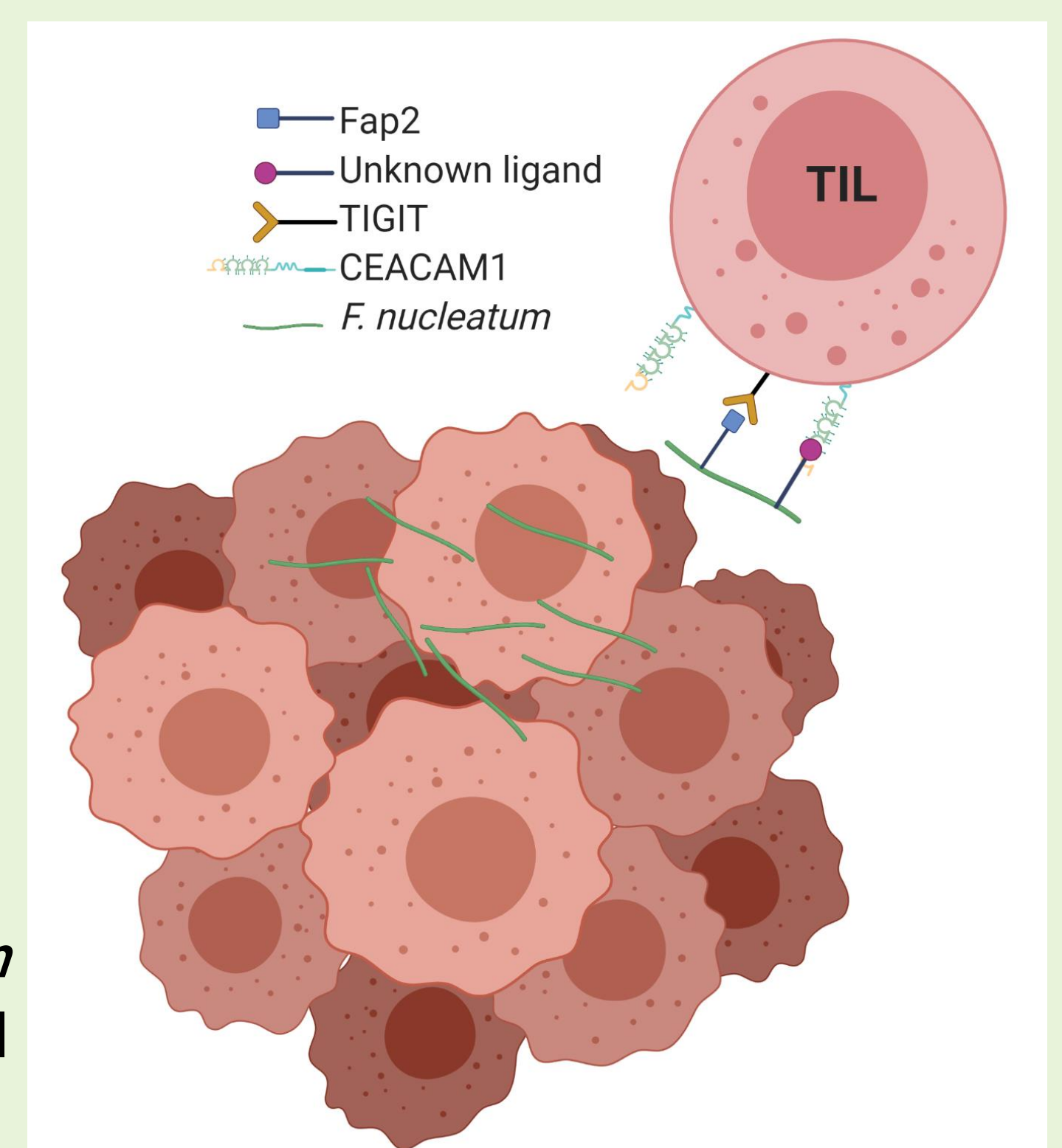

Figure 5. Schematic representation of the Fap2-dependent and Fap2-independent mechanisms to inhibit TILs activities via TIGIT and CEACAM1 inhibitory receptors.

CONCLUSIONS

The involvement of *F. nucleatum* in CRC carcinogenesis is still at the stage of association. Yet, in this review, three examples of *F. nucleatum* possible oncogenic mechanisms have been discussed and could be considered the three axis of *F. nucleatum* role in CRC progression. However, these mechanisms surely demonstrate just a glimpse of the diverse ways by which *F. nucleatum* could promote a protumourigenic environment. Hence, findings to date are not enough to determine with certainty the clinical prognosis of *F. nucleatum* – positive CRC cases. Thus, robust experimental approaches, be it within human cohorts or preclinical models, along with reproducible results across microbiota studies are crucial to bridge the translational gap, and to ensure that data are neither lost in translation nor mistranslated clinically.

Relevant references

[1] Komiya, Y. *et al*. Patients with colorectal cancer have identical strains of *Fusobacterium nucleatum* in their colorectal cancer and oral cavity. *Gut* 68, 1335–1337 (2019). [2]. Abed, J. *et al*. Fap2 Mediates *Fusobacterium nucleatum* Colorectal Adenocarcinoma Enrichment by Binding to Tumor-Expressed Gal-GalNAc. *Cell Host Microbe* 20, 215–225 (2016). [3]. Kostic, A. D. *et al*. *Fusobacterium nucleatum* Potentiates Intestinal Tumorigenesis and Modulates the Tumor-Immune Microenvironment. *Cell Host Microbe* 14, 207–215 (2013). [4]. Fardini, Y. *et al*. *Fusobacterium nucleatum* adhesin FadA binds vascular endothelial cadherin and alters endothelial integrity. *Mol. Microbiol*. 82, 1468–1480 (2011). [5]. Rubinstein, M. R. *et al*. *Fusobacterium nucleatum* Promotes Colorectal Carcinogenesis by Modulating E-Cadherin/β-Catenin Signaling via its FadA Adhesin. *Cell Host Microbe* 14, 195–206 (2013). [6]. Rubinstein, M. R. *et al*. *Fusobacterium nucleatum* promotes colorectal cancer by inducing Wnt/β-catenin modulator Annexin A1 . *EMBO Rep*. 20, 1–17 (2019). [7]. Gur, C. *et al*. Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* 42, 344–355 (2015). [8]. Gur, C. *et al*. *Fusobacterium nucleatum* supresses anti-tumor immunity by activating CEACAM1. *Oncoimmunology* 8, 1–6 (2019). *All figures were created with https://biorender.com/*