

# Immunohistochemical expression of cannabinoid receptors in women's cancers: what's new?

Luca Roncati<sup>1,\*</sup>, Maria Vadalà<sup>2</sup>, Veronica Corazzari<sup>2</sup>, Beniamino Palmieri<sup>2</sup>

<sup>1</sup>Department of Maternal, Child and Adult Medical and Surgical Sciences, University Hospital of Modena, 41124 Modena, Italy

<sup>2</sup>Second Opinion Network, 41100 Modena, Italy

\*Correspondence: [luca.roncati@unimore.it](mailto:luca.roncati@unimore.it); [roncati.luca@aou.mo.it](mailto:roncati.luca@aou.mo.it); [emailmedical@gmail.com](mailto:emailmedical@gmail.com) (Luca Roncati)

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The cannabinoid receptors belong to the G protein-coupled receptor superfamily and are integral part of the endocannabinoid system. Two main types of cannabinoid receptors are known: cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). In the last few years, great attention has been paid to the immunohistochemical evaluation of CB1 and CB2 expression in various types of tumors, including women's cancers, for the alleged anticancer properties of cannabinoids. Today, in the modern era of precision oncology, monoclonal antibodies for the immunohistochemical evaluation of CB1 and CB2 expression are available on the market; therefore, our recommendation is to submit preliminary the formalin-fixed, paraffin-embedded, bioptic or surgical specimen of neoplastic tissue, containing at least 100 tumor cells and coming from the selected patient with no history of cannabis abuse, to predictive immunohistochemistry, before undertaking any cannabinoid-based therapeutic attempt, in association with conventional anticancer treatments or when the most advanced care is failing. The receptor expression is determined through a 'tumor proportion score' (TPS), which represents the percentage of viable neoplastic cells showing partial or complete membrane staining. By exploiting a methodology analogous to that applied for PD-L1 (programmed death-ligand 1) testing on cancer tissues, the specimen can be considered to have a high CB1 and/or CB2 expression if  $TPS \geq 50\%$ ; a value between 1--49% corresponds to a low expression, while below 1% certifies no significant expression and, thus, no eligibility to a cannabinoid-based pharmacological approach.

## Keywords

Cannabinoid receptors; Cannabinoid receptor 1 (CB1); Cannabinoid receptor 2 (CB2); Immunohistochemistry; Predictive immunohistochemistry; Tumor proportion score (TPS); Cancer

The cannabinoid receptors are G protein-coupled receptors, also known as serpentine receptors or heptahelical receptors because they pass through the cytoplasmic membrane seven times before coupling with G protein inside the cell (Fig. 1). They are able to bind with three major groups of extracellular ligands: endocannabinoids (e.g., anandamide, 2-arachidonoylglycerol), physiologically produced and released in the body as neurotransmitters; phytocannabinoids (e.g., tetrahydrocannabinol, cannabidiol), found numerous in

cannabis, one of the fundamental herbs in traditional Chinese medicine (Fig. 2); and synthetic cannabinoids, manufactured in laboratory, such as HU-210 (Hebrew University 210, from the homonymous Israeli university where it was first synthesized), about 100 times as potent as tetrahydrocannabinol, and HU-331, a potential anticancer drug which inhibits DNA topoisomerase II even at nanomolar concentrations [1, 2]. The endocannabinoid receptors are integral part of the endocannabinoid system, a biological system involved in several circuits including appetite, insulin sensitivity, energy balance, analgesia, memory, immunity, exercise-induced euphoria, intestinal motility, mood, sleep, thermoregulation, fertility and pregnancy [3]. For instance, the analgesic, anticonvulsant and thermoregulatory effects of paracetamol are due to its active metabolite N-arachidonoylaminophenol (AM404), now considered an endocannabinoid system enhancer [4]. At present, two main types of cannabinoid receptors are known: cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). They show an amino acid similarity around 44%; however, when only the transmembrane domains are considered, the amino acid similarity reaches about 68% [5]. Main target of anandamide and tetrahydrocannabinol, CB1 is primarily localized in the central nervous system, but also in the lungs, liver, kidneys, digestive tract, retina, reproductive organs and placenta; it has also been noted to form a functional heterodimer together with the orexin receptor 1, which regulates feeding behavior and stress/pressor responses [6, 7]. In fact, CB1 distribution in the endocannabinoid system is frequently overlapping with the orexinergic projections, that mediate many of the same functions, both physical and cognitive [8, 9]. CB2 is present in the peripheral nervous system and, predominantly, in the immune/hematopoietic cells; however, recent studies have demonstrated its existence in regions of the brain as well [10]. It represents the preferential binding site for 2-arachidonoylglycerol and cannabidiol [11]. Besides to CB1 and CB2, certain orphan receptors, nicknamed non-CB1/CB2, have been found to bind endocannabinoids, such as GPR18 (G protein-coupled receptor 18), GPR55 and GPR119, expressed on the endothelial and smooth muscle cells and in the central nervous system, too

[12]. In particular, GPR55 has been suggested as possible CB3 [13].

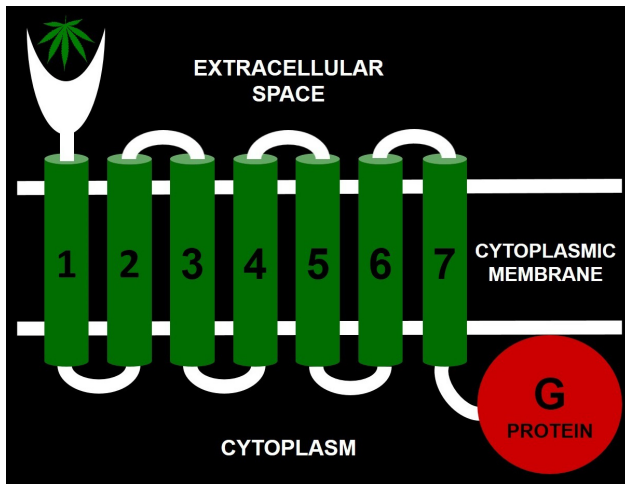


Fig. 1. Structural schema of a cannabinoid receptor: the extracellular portion, able to bind the cannabinoid (here depicted as a cannabis leaf) is connected with the G protein, inside the cell, through seven transmembrane domains, green colored, hence the superfamily name of ‘seven-(pass)-transmembrane domain receptors’.

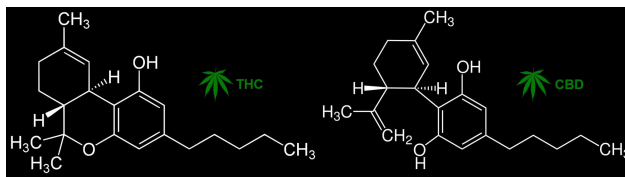


Fig. 2. Structural formulas of tetrahydrocannabinol (THC, on the left) and cannabidiol (CBD, on the right): although quite similar, the former is psychoactive while the latter is devoid of relevant psychoactivity, feature that makes it the most manageable for therapeutic purposes (e.g., chemotherapy-related nausea and vomiting, drug-resistant epilepsy, multiple sclerosis, neurodegenerative disorders, anorexia, spasticity, neuropathic pain, arthritis, fibromyalgia, inflammatory bowel diseases, overactive bladder, glaucoma).

In the last few years, great attention has been paid to the immunohistochemical detection of cannabinoid receptors in various types of tumors, in particular malignant gliomas for the notorious capability of cannabinoids to easily overcome the blood-brain barrier, but also in women’s cancers [14]. In 2015, a Chinese research group has ascertained that CB2 overexpression induces the apoptosis of cervical carcinoma Caski cells, disclosing new important scenarios [15]. In parallel, some European authors have immunohistochemically evaluated the CB1 and CB2 expression in endometrial carcinoma obtaining mixed results, but concluding that they can be considered therapeutic targets to be exploited, if significantly present [16, 17]. Moreover, the CB1 expression has been reported to increase from benign and borderline to ma-

lignant ovarian epithelial tumors [18]. As well-known, selective estrogen receptor modulators (SERMs) are commonly used to treat estrogen receptor-positive breast cancer; however, tamoxifen and newer classes of SERMs exhibit cytotoxicity against estrogen receptor-negative cancers, suggesting a non-estrogenic mechanism of action [19]. Surprisingly, this mechanism has been traced back to the endocannabinoid system, since tamoxifen has been proven to behave like a cannabinoid inverse agonist, binding CB1 and CB2, so resulting a promising scaffold for novel drug development [20, 21]. In the English medical literature, the power of cannabinoids to inhibit the growth and migration of breast cancer cell lines has been described in depth [22, 23]. CB2 activation suppresses tumor cells by inhibiting the epidermal and insulin growth factor receptor pathways and CB2 is widely considered a pivotal regulator of the epidermal growth factor receptor 2 (Her2) pro-oncogenic signaling in female breast cancer [24, 25]. Moreover, the CB2-Her2 heteromer has been recently discovered, providing a novel antitumor target in Her2-positive breast cancers [26]. Today, in the modern era of precision oncology [27, 28], monoclonal antibodies for the immunohistochemical evaluation of CB1 and CB2 expression are available on the market; therefore, our recommendation is to submit preliminary the formalin-fixed, paraffin-embedded, biptic or surgical specimen of neoplastic tissue, containing at least 100 tumor cells and coming from the selected patient with no history of cannabis abuse, to predictive immunohistochemistry, before undertaking any cannabinoid-based therapeutic attempt, in association with conventional anticancer treatments or when the most advanced care is failing. The receptor expression is determined through a ‘tumor proportion score’ (TPS), which represents the percentage of viable neoplastic cells showing partial or complete membrane staining [29]. By exploiting a methodology analogous to that applied for PD-L1 (programmed death-ligand 1) testing on cancer tissues [30], the specimen can be considered to have a high CB1 and/or CB2 expression if  $TPS \geq 50\%$ ; a value between 1–49% corresponds to a low expression, while below 1% certifies no significant expression and, thus, no eligibility to a cannabinoid-based pharmacological approach.

### Author contributions

LR conceived, designed and supervised the study, performed the experiments, interpreted the data, prepared the figures with the related legends, and wrote the manuscript; MV analyzed the data; VC performed the literature search; BP contributed reagents and materials.

### Ethics approval and consent to participate

Not applicable.

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## Conflict of interest

The authors declare no conflict of interest.

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