



# ***Tubulin detyrosination: clinically useful markers of tumor prognosis anti Glu-Tubulin anti $\Delta$ 2-Tubulin***

**AbCys proposes 2 specific antibodies against Glu and  $\Delta$ 2-tubulin.** These polyclonal rabbit antibodies can be used to perform Immunochemistry (paraffin and frozen sections), immunofluorescence, western Blot and immunoprecipitation.

## **I. Tubulin modifications and Cancer.**

Microtubules are major cytoskeletal structures, centrally involved in the control and mechanics of cell division, being the principal components of the mitotic spindle in eukaryotic cells. The building block of microtubules is the tubulin heterodimer. Tubulin is subject to specific post translational modifications including a cycle of tyrosine removal and addition at the COOH terminus of the  $\alpha$  subunit (see figure 1). This cycle involves two enzymes, TTL (**Tyrosine, Tubulin, Ligase**) and TCP (**Tubulin CarboxyPeptidase**), and generates two major forms of tubulin: tyrosinated tubulin (Tyr-tubulin) and detyrosinated (Glu-tubulin). A third tubulin species ( $\Delta$ 2-tubulin) arises by removal of the COOH-terminal Glu residue from the  $\alpha$  chain of Glu-tubulin.

Tyr-tubulin is the dominant tubulin species in cycling cells. Glu-tubulin is abundant in neurons but can be present in stable microtubules of other cell types.  $\Delta$ 2-tubulin normally has high neuronal specificity. However, abnormal accumulation of Glu-tubulin and  $\Delta$ 2-tubulin in cancer cells of both fibroblastic and epithelial origin, was observed during tumor growth in nude mice.

This accumulation is attributable to TTL suppression and apparently represents a strong selective advantage for cancer cells.

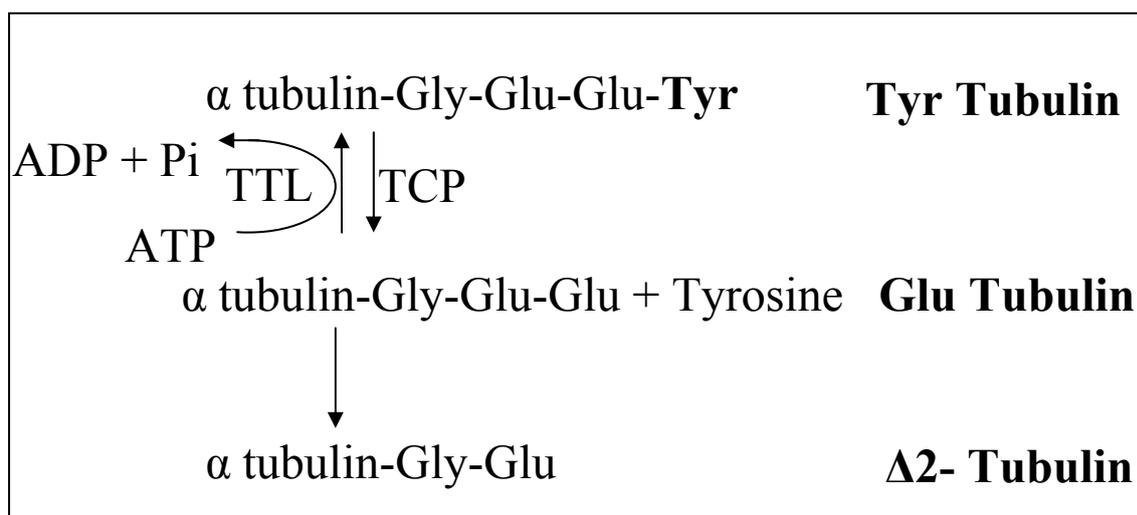


Figure 1: detyrosination/tyrosination cycle

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These results led Dr Lafanechère, researcher from the CEA of Grenoble, to develop two specific antibodies against the forms Glu and  $\Delta$ 2 of the tubulin.

These antibodies were used in a study of the occurrence and the significance of tubulin detyrosination in human breast tumors in collaboration with the Anticancerous Centre Léon Bérard of Lyon.

A total of 134 breast cancer tumors from patients with or without known complications were studied over a follow-up period of  $31 \pm 10$  months.

The mean age of the patients at the time of diagnosis was 57 years. For each patient, detailed data concerning the histology and extension of the tumor were available.

Tumor cells containing detyrosinated tubulin were visualized by immunohistochemical staining of paraffin-embedded tissue sections (see figure 2 from reference 4).

Cancer cells with detyrosinated tubulin were observed in 53% of the tumors and were predominant in 19.4% of the tumors. Tubulin detyrosination correlated to a high degree of significance ( $P < 0.001$ ) with a high Scarf-Bloom-Richardson (SBR) grade, a known marker of tumor aggressiveness. Among SBR grade 1 tumors, 3.8% were strongly positive for tubulin detyrosination compared with 65.4% of the SBR grade 3 tumors.

The SBR component showing the strongest correlation with tubulin detyrosination was the mitotic score. In the entire patient population, neither the SBR grade nor the detyrosination index had significant prognostic value ( $P = 0.11$ ,  $P = 0.27$ , respectively), whereas a combined index was significantly correlated with the clinical outcome ( $P = 0.02$ ).

A preliminary subgroup analysis indicated that tubulin detyrosination may define high and low-risk groups in breast cancer tumors with an SBR grade of 2.

**This study shows that tubulin detyrosination is a frequent occurrence in breast cancer, easy to detect, and linked to tumor aggressiveness.**

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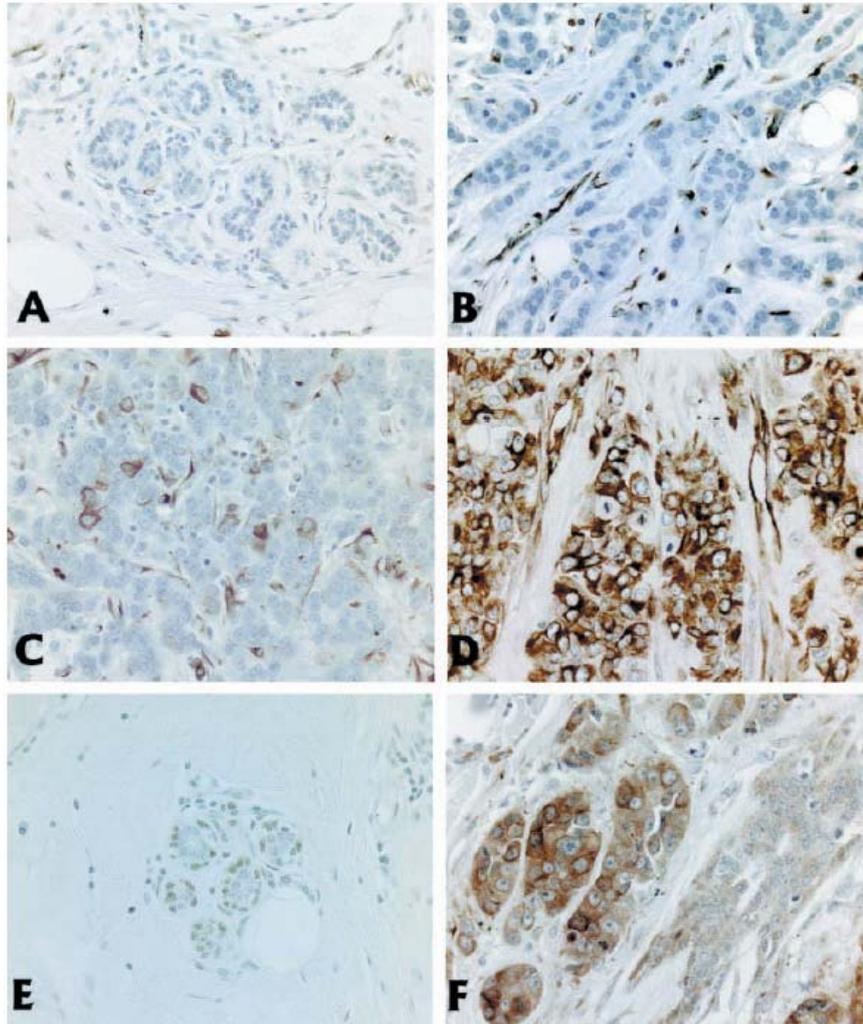


Figure 2: Immunohistochemical staining of Glu- and  $\Delta 2$ -tubulin in breast tumor tissue. Paraffin-embedded breast tissue sections were stained for Glu-tubulin (A–D) or  $\Delta 2$ -tubulin (E and F). Sections were counterstained with Harris' hematoxylin. Examples of immunostaining in normal areas of breast tissue are shown for Glu-tubulin (A) and for  $\Delta 2$ -tubulin (E), respectively. Nuclei are stained in blue by hematoxylin, and Glu or  $\Delta 2$ -tubulin is stained in brown. Examples of tumors scored as negative (grade 1), positive (grade 2), or strongly positive (grade 3) for Glu-tubulin staining are shown in B, C, and D, respectively. A tumor strongly positive for  $\Delta 2$ -tubulin is shown in F.

These results have recently been extended to other tumor types, including lung cancer (5) and neuroblastoma (6). Thus, TTL suppression and resulting tubulin detyrosination in human cancers is associated with increased tumor aggressiveness.

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## **II. Datasheets**

### **Anti Glu-Tubulin (Anti Glu-Tub)**

Isotype : IgG

Size : [ 300  $\mu$ g, lyophilized]

Cat.N : AbC0101

Description : Tubulin, the dimeric subunit of microtubules, is submitted to post translational modifications including cyclic removal and addition of tyrosine at the COOH terminus of the alpha subunit. Anti Glu-Tub polyclonal antibody recognizes specifically detyrosinated form of the tubulin alpha chain containing the EEGEE carboxy-terminal sequence (see Ref 1 and 2)

Source : Rabbit

Immunogen : A peptide corresponding to the seven C-terminal amino acids of Glu tubulin ie GEEEGEE

Product : Purified IgG, 300  $\mu$ g lyophilized, for 100  $\mu$ l reconstituted in PBS.

Specificity : The antibody works in all mammals, sea urchin, plants, not in yeast (Ref 3).

Applications :

•IHC and IF : For immunohistochemistry and electron microscopy. tissue sections either frozen, paraffin included or fixed with any kind of fixative (Ref 4), 1:500 dilution is recommended.

•WESTERN-BLOT : 1 :1000 dilution is recommended

IMMUNOPRECIPITATION : not tested

Storage : Store at 4°C after reconstitution,

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## **Anti $\Delta$ 2-Tubulin (Anti $\Delta$ 2-Tub)**

Isotype : IgG

Size : [ 300  $\mu$ g lyophilized]

Cat.N : AbC0102

Description : Tubulin, the dimeric subunit of microtubules, is submitted to post translational modifications including cyclic tyrosine removal and addition at the COOH terminus of the alpha subunit. The cycle involves two enzymes, TTL (Tubuline Tyrosine Ligase) and Tubulin carboxypeptidase, and generates two major forms : tyrosinated tubulin and detyrosinated tubulin (Glu-tubulin). Removal of the COOH-terminal Glu residue from the alpha chain of Glu-tubulin generates the  $\Delta$ 2-tubulin form. The anti- $\Delta$ 2-Tub polyclonal antibody recognizes specifically the delta 2 form of the tubulin alpha chain containing the EEGE carboxy-terminal sequence (Ref 1).

Source : Rabbit

Immunogen : A peptide corresponding to the seven C-terminal amino acids of  $\Delta$ 2-tubulin i.e. EGEEEGE

Product : Purified IgG, 300  $\mu$ g to be reconstituted in 100  $\mu$ l of PBS

Specificity : The antibody works in all mammals, sea urchin, plants, not in yeast (Ref 3).

Applications :

•IHC and IF : For immunohistochemistry and electron microscopy. tissue sections either frozen, paraffin included or fixed with any kind of fixative (Ref 4), 1:500 dilution is recommended.

•WESTERN-BLOT : 1 :1000 dilution is recommended.

IMMUNOPRECIPITATION : [ not tested]

Storage : Store at 4°C after reconstitution,

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### **III. References**

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