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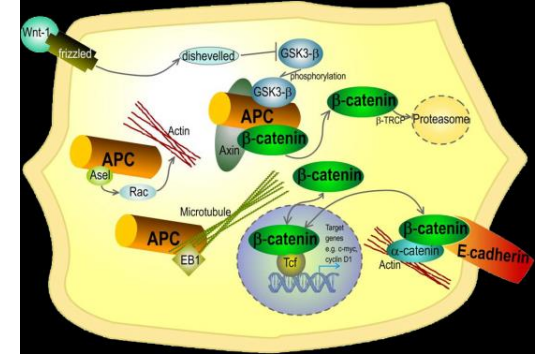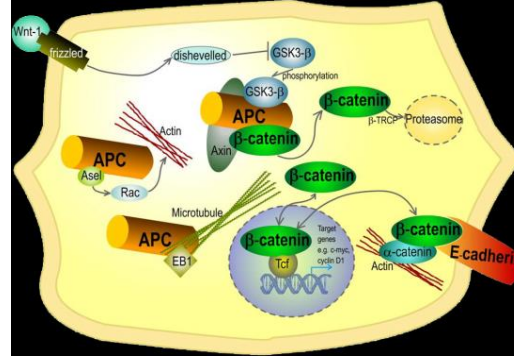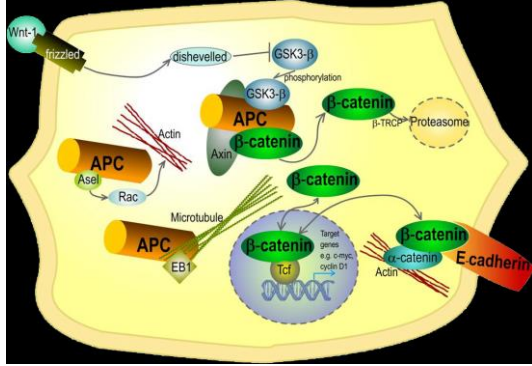UNIVERSITY OF ZAGREB MEDICAL SCHOOL
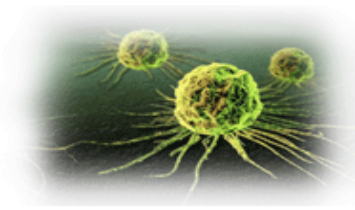
CROATIAN INSTITUTE FOR BRAIN RESEARCH

MOLECULAR CHANGES OF WNT SIGNALING IN ASTROCYTIC TUMOR ETIOLOGY

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- The Wnt signaling pathway, which was discovered 30 years ago is one of the basic cellular pathways.

- Wnt signaling is essential during the development of the central nervous system because it behaves as a regulator of the embryonic cell patterning, proliferation, differentiation, cell adhesion, cell survival and apoptosis.

- Processes that include cellular adhesion and synaptic rearrangements require the expression of molecular components of the wnt pathway.

- The malfunctioning of wnt signaling pathway is responsible for tumorigenesis in many different tissues and lately it was implicated in brain tumorigenesis as well.
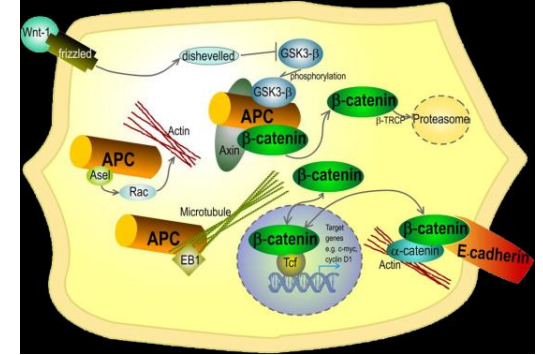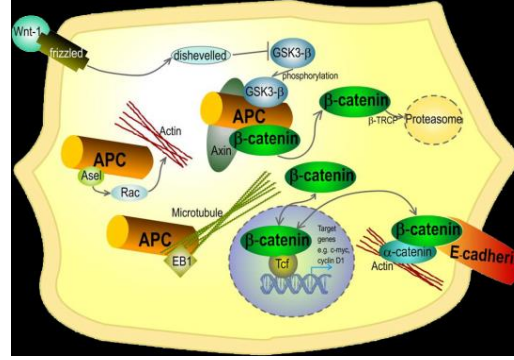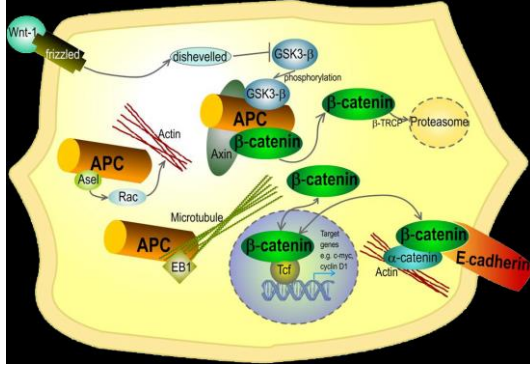
- Molecular and genetic landscapes of human astrocytic brain tumors need elucidation.

- Our group is studying Wnt signaling in glial branch of brain tumors. The investigation of key players of wnt signaling, beta-catenin (CTNNB1), TCF1 and LEF1, adenomatous polyposis coli (APC) and axin (AXIN1) in a set of human astrocytic brain tumors is going to be presented.

- The main signaling molecule of the pathway is β-catenin, but APC and AXIN1 are critical component of its destruction machinery. TCF1 and LEF1 are transcription factors of the pathway.
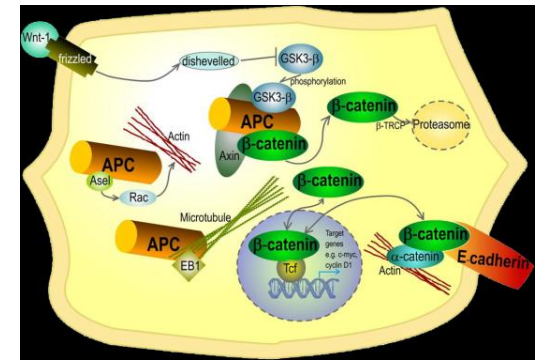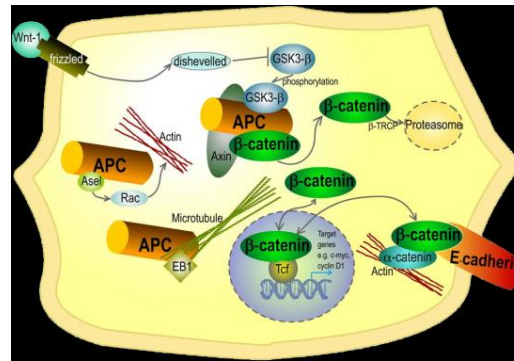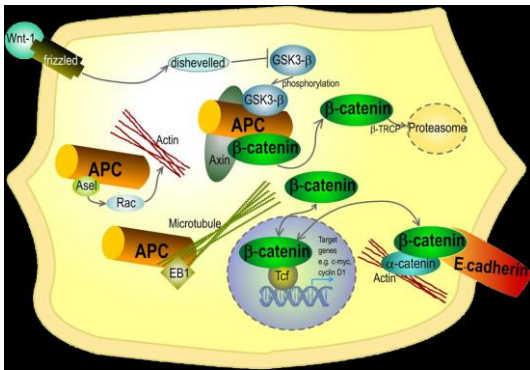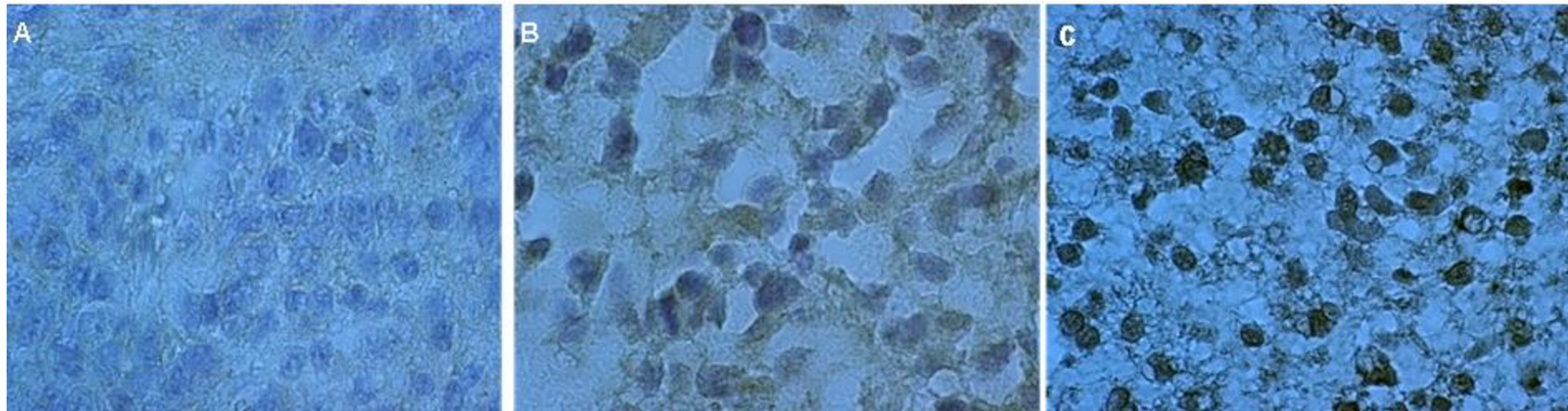
- beta-catenin (CTNNB1)

- TCF1 and LEF1

- adenomatous polyposis coli (APC)

- axin (AXIN1)

CTNNB1

The results on beta-catenin protein levels demonstrated that 50% of glioblastomas (WHO grade IV) and 56% of astrocytomas (WHO grades II and III) showed upregulation of beta-catenin and nuclear localization was found in 52.1% of glioblastomas.

Astrocytic brain tumors immunohistochemically stained for protein expression of beta-catenin. (A) Glioblastoma sample negative for the expression of beta-catenin proteins. (B) Glioblastoma sample with cytoplasmic localization of beta-catenin and glioblastoma sample with cytoplasmic and nuclear localization of beta-catenin (C).

CTNNB1

The investigation of beta-catenin demonstrated 10% of samples with potential activating mutations. We targeted exon 3 of CTNNB1 gene since it has been reported as mutational hot spot.
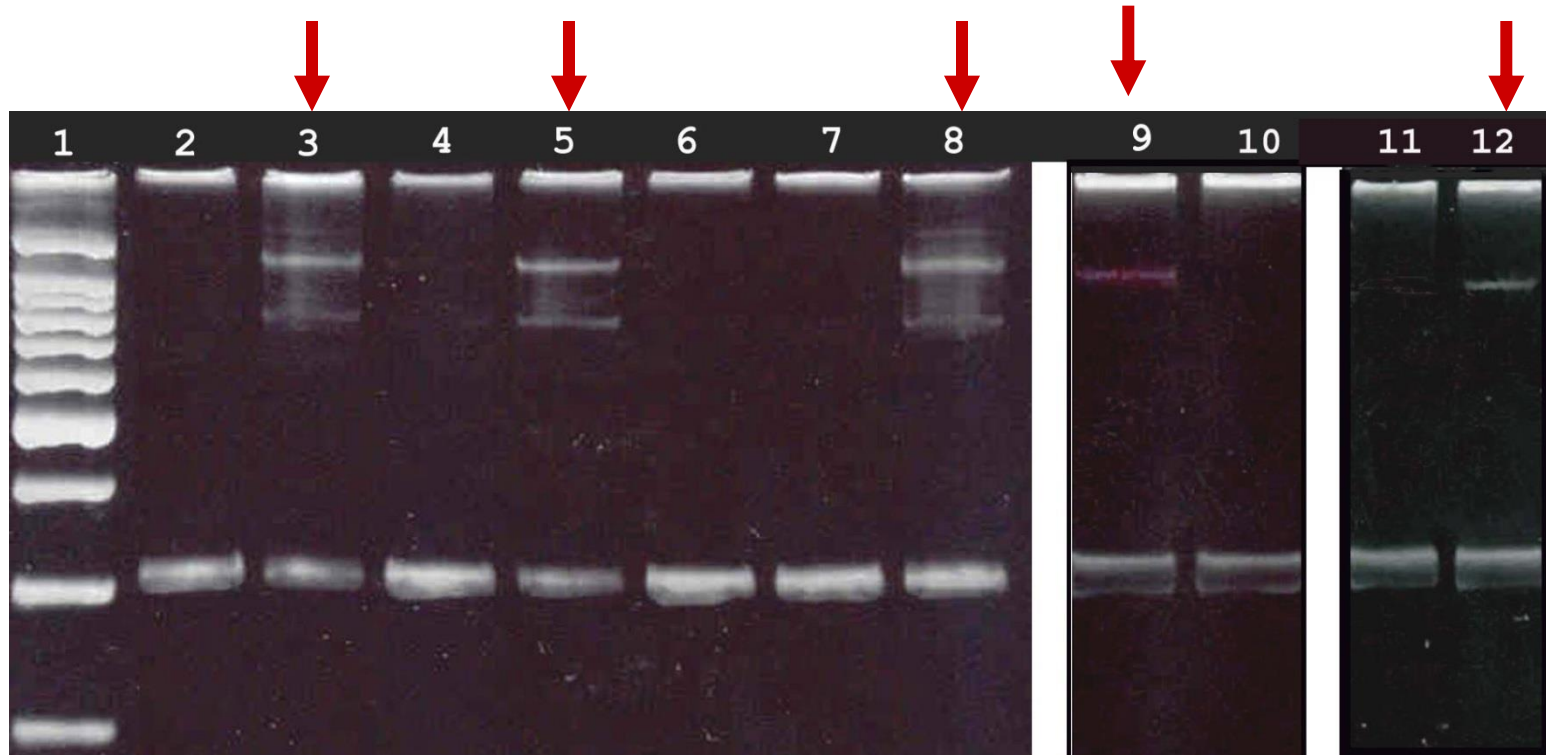
Heteroduplex analysis of the astrocytic brain tumors . Exon 3 of the CTNNB1 gene was screened for mutations on GMA gels (Elchrom Scientific). Lane 1 - M3 standard; lanes 3, 5, 8, 9, 12 -additional bands showing heteroduplexes when tumor and normal samples are mixed; lanes 2, 4, 7, 10, 11- corresponding blood DNA samples; lane 6 -tumor DNA sample.

TCF1 and LEF1

The expression of the both transcription factors was found to be present in the majority of the analyzed samples and heterogeneously distributed throughout the tumors with nuclear localizations, suggesting increased transcriptional activity. Immunostaining of LEF-1 and TCF-1 proteins is demonstrated.
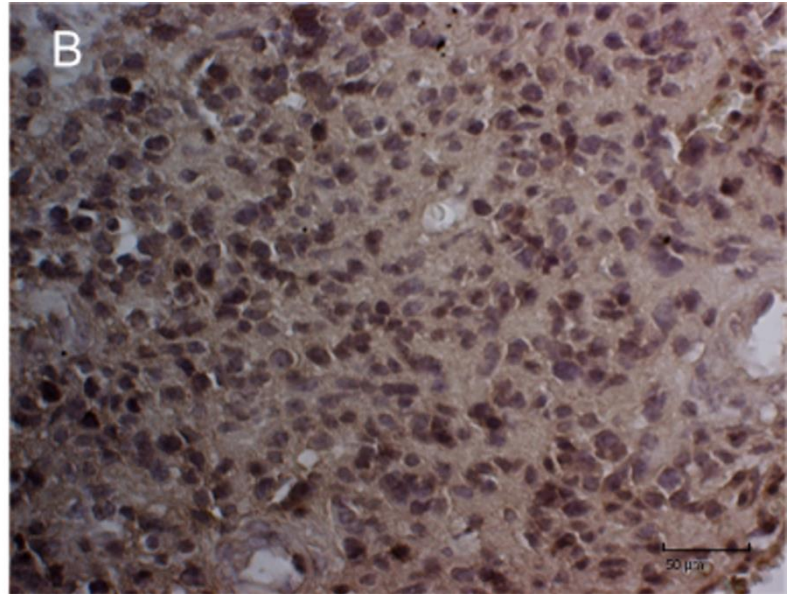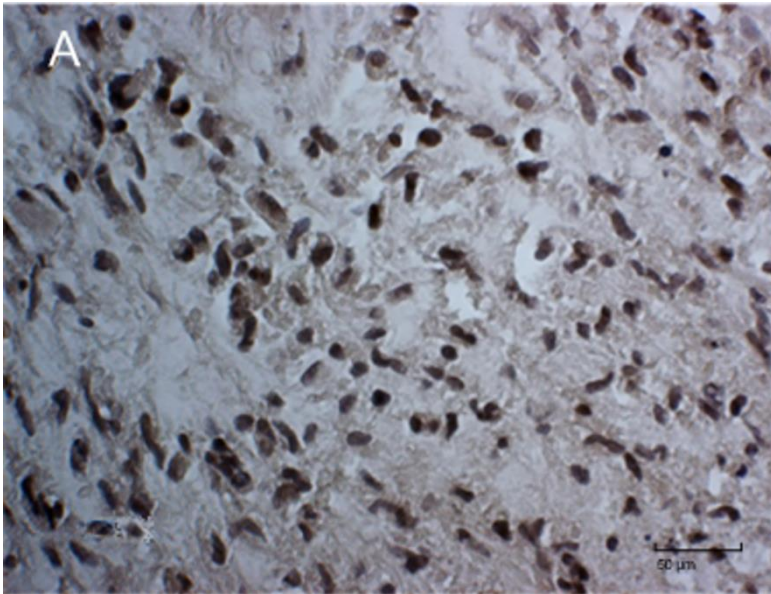
Characteristic immunohistochemical staining of strong TCF1 (A) and LEF1 (B) expression in glioblastomas.

APC

Allelic losses of APC gene were frequent with glioblastomas showing 60% and diffuse astocytomas (grade II) 20% of LOHs.
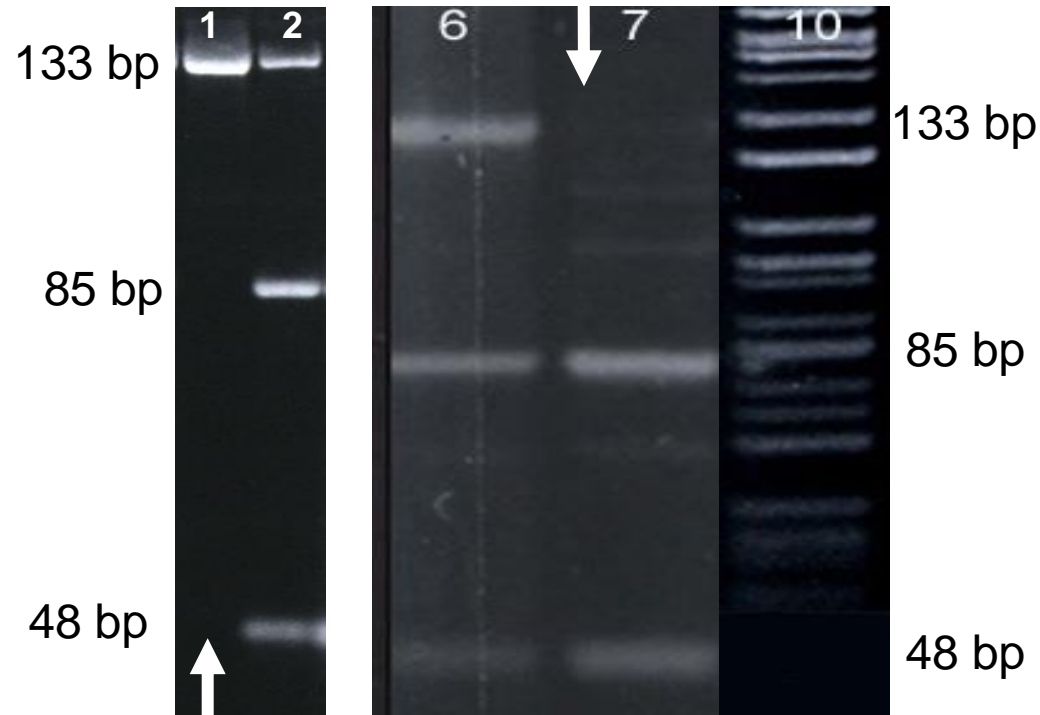
Loss of heterozygosity of APC gene in glioblastoma sample. Exon 11/RsaI/RFLP is demonstrated. Lane1 LOH in glioblastoma (the digested/cut allele is missing); lane 2 corresponding heterozygous blood sample; lane 6- heterozygous blood sample; lane 7- LOH in corresponding glioblastoma (the undigested/uncut allele is missing); lane 10 - standard M3 (Elchrom scientific).
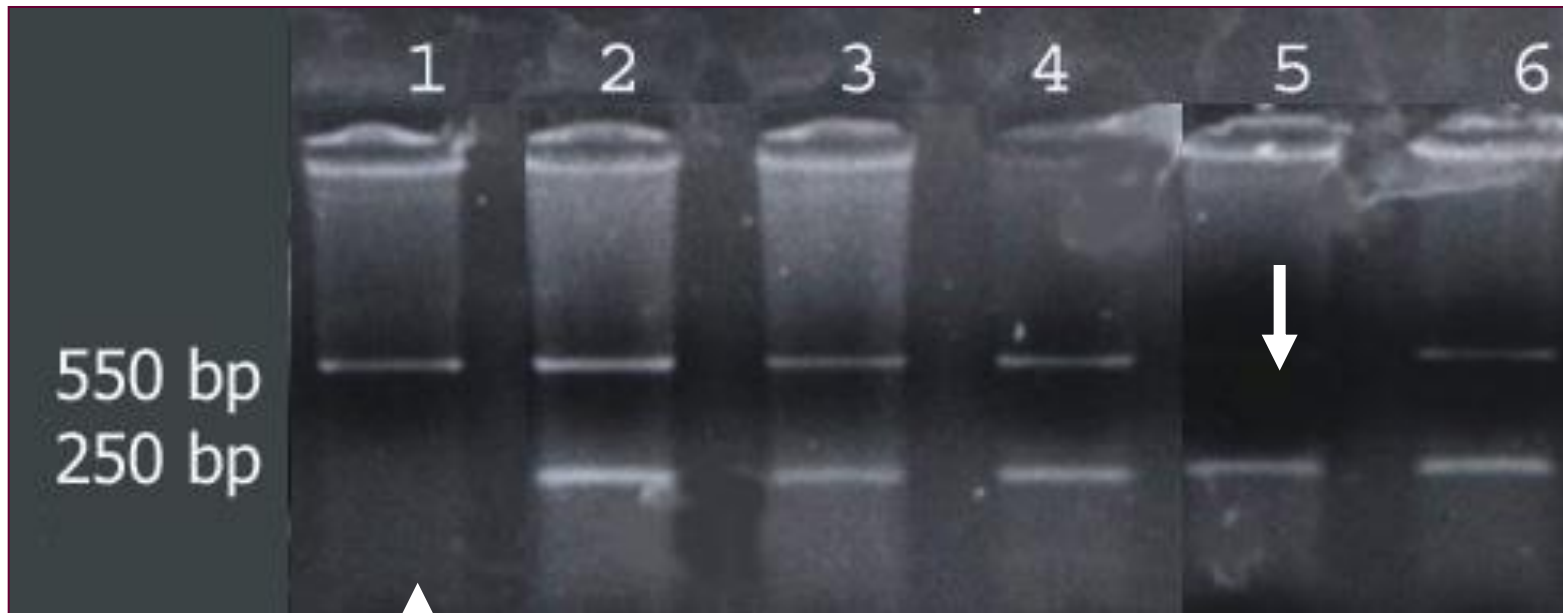
APC

Allelic losses of APC gene were frequent with glioblastomas showing 60% and diffuse astocytomas (grade II) 20%.

**Loss of heterozygosity of APC gene in 2 patients with glioblastoma. Exon 15/MspI/RFLP is demonstrated. Lanes 1, 5 - LOHs in glioblastoma samples; lanes 2, 6 – corresponding heterozygous blood samples; lanes 3, 4 - heterozygous samples, both alleles, cut and uncut, are visible. One allele is deleted, and the remaining one might have suffered another type of mutation, probably point mutation or a small deletion.**

Axin1

Tumor suppressor gene AXIN1, 16p13.3, 96 kDa, is an inhibitor of wnt signaling. It down-regulates beta-catenin by facilitating its phosphorylation by GSK3-beta. It binds directly to APC, beta-catenin, GSK3-beta and Dishevelled (Cliffe et al. (2003) Curr Biol 13:960–966; Cong F and Varmus H. (2004) Proc Natl Acad Sci USA 101:2882-2887; Chen et al., (2013), IUBMB Life65-. 685-681).
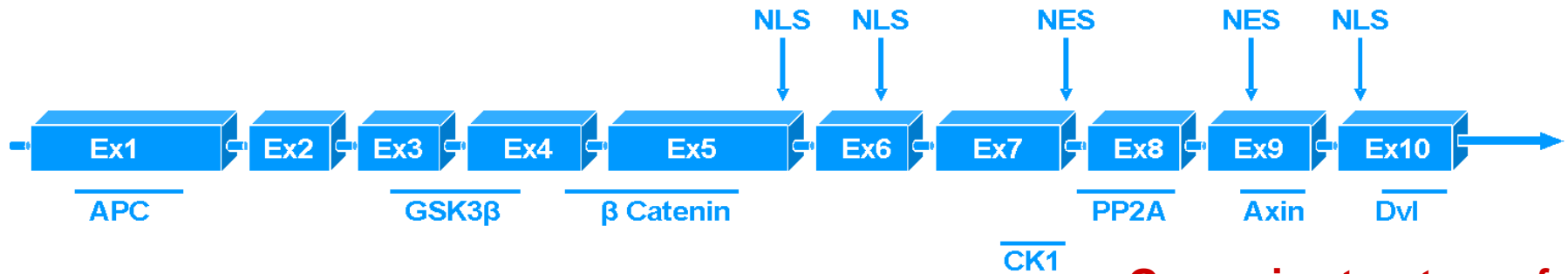
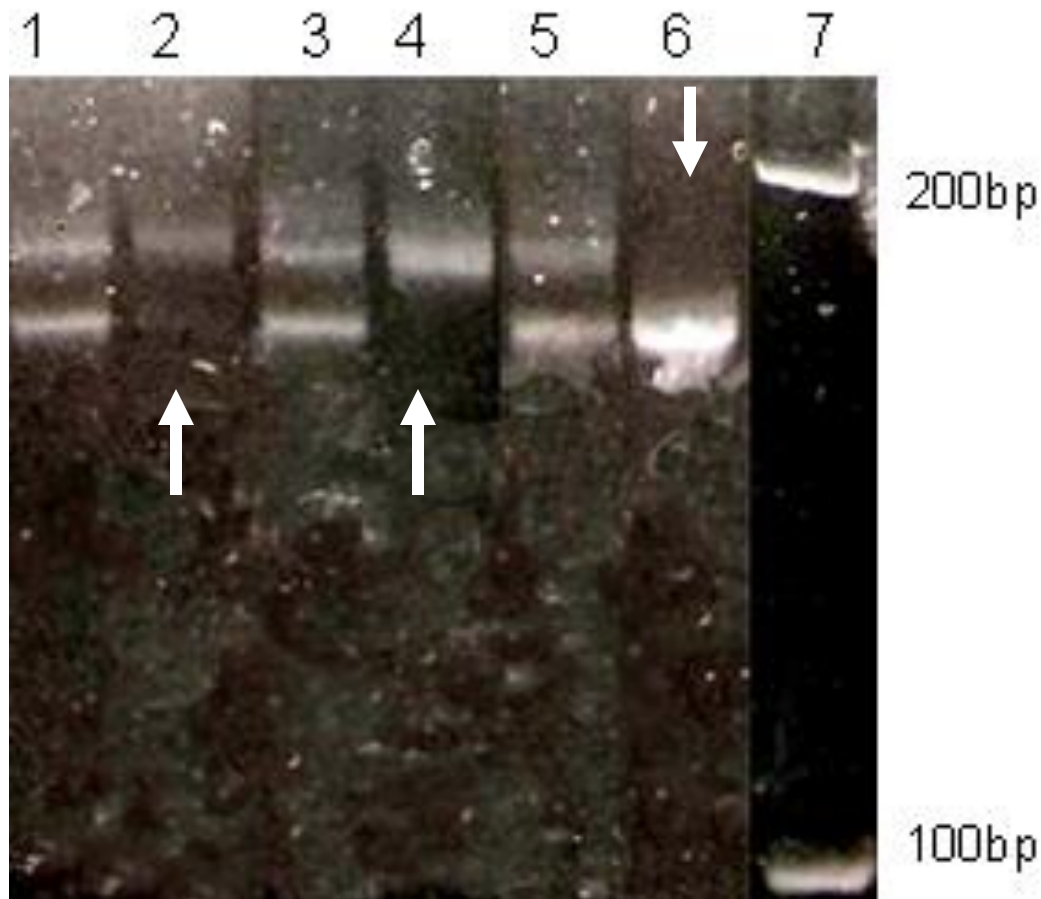**Genomic structure of Axin 1. Axin 1 is composed of 10 exons and they encode isoform a, while in isoform b exon 8 is spliced out.**
Figure 2. Two crystallized domains of the Axin 1 protein are shown: (A) RGS and (B) DIX.
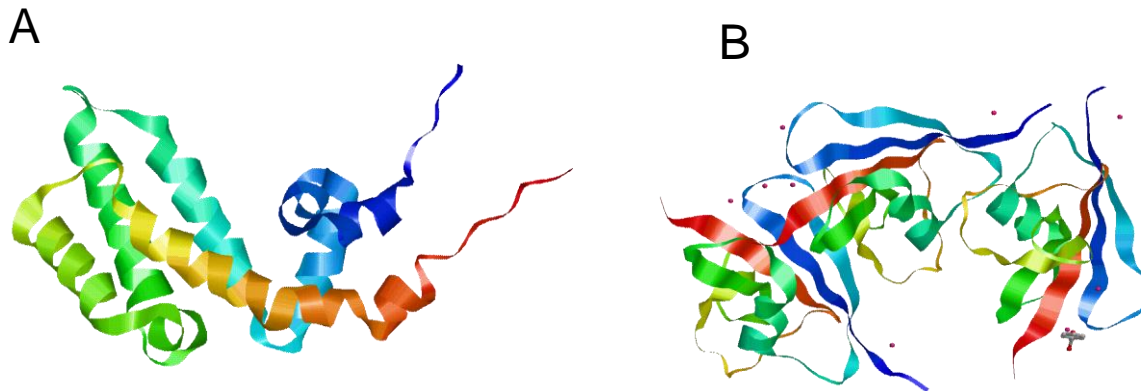
Results: Axin1

Allelic losses of AXIN1 were found in 10% of glioblastomas.

Loss of heterozygosity of AXIN1 gene in 3 glioblastomas, lanes 2, 4 and 6 LOHs, lanes 1, 3, 5 –correspondig blood DNA

Axin1

In 31% of glioblastomas and 22% of astrocytomas intensive downregulation of axin1 proteins was detected. In 31% of glioblastomas axin1 was localized in the nucleus.

A

B
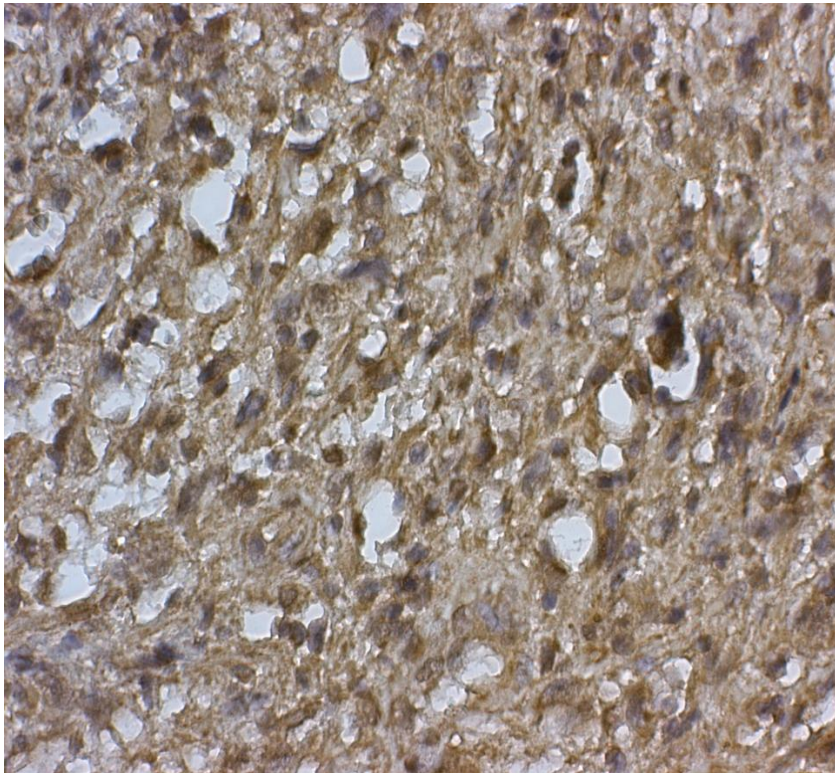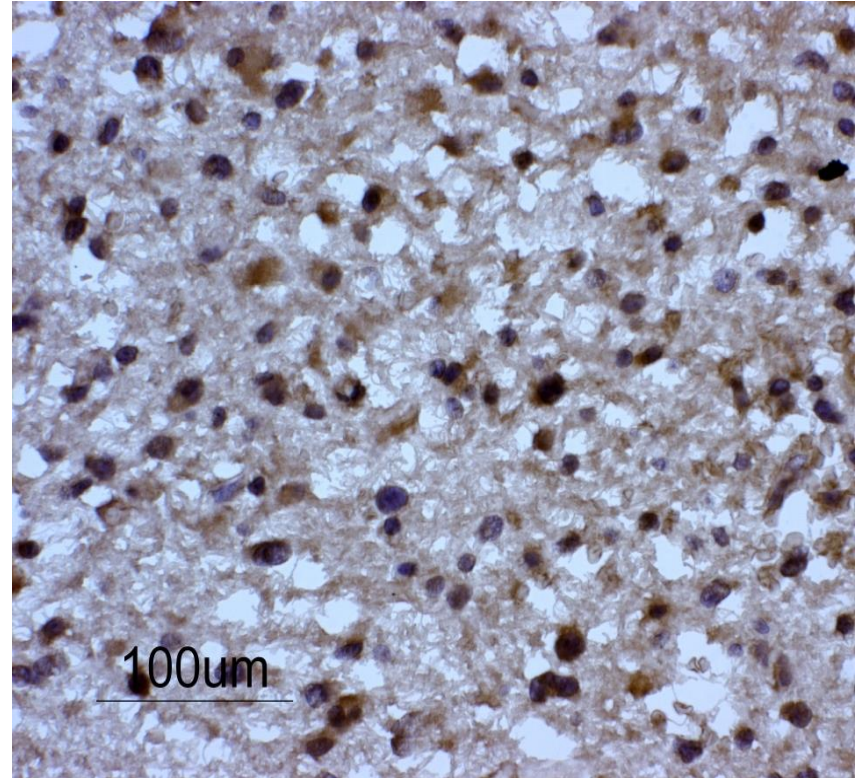
Glioblastoma samples immunohistochemically stained for protein expression of axin1. (A) Cytoplasmic localization of axin1 and (B) nuclear localization of axin1.

Cellular localization of Axin1 in astrocytomas

Legend:
- cytoplasm
- cytoplasm and nucleus
- nucleus
- no expression

Data values: 66,7% (cytoplasm), 21,6% (cytoplasm and nucleus), 11,7% (nucleus), 0% (no expression)

AXIN1&CTNNB1

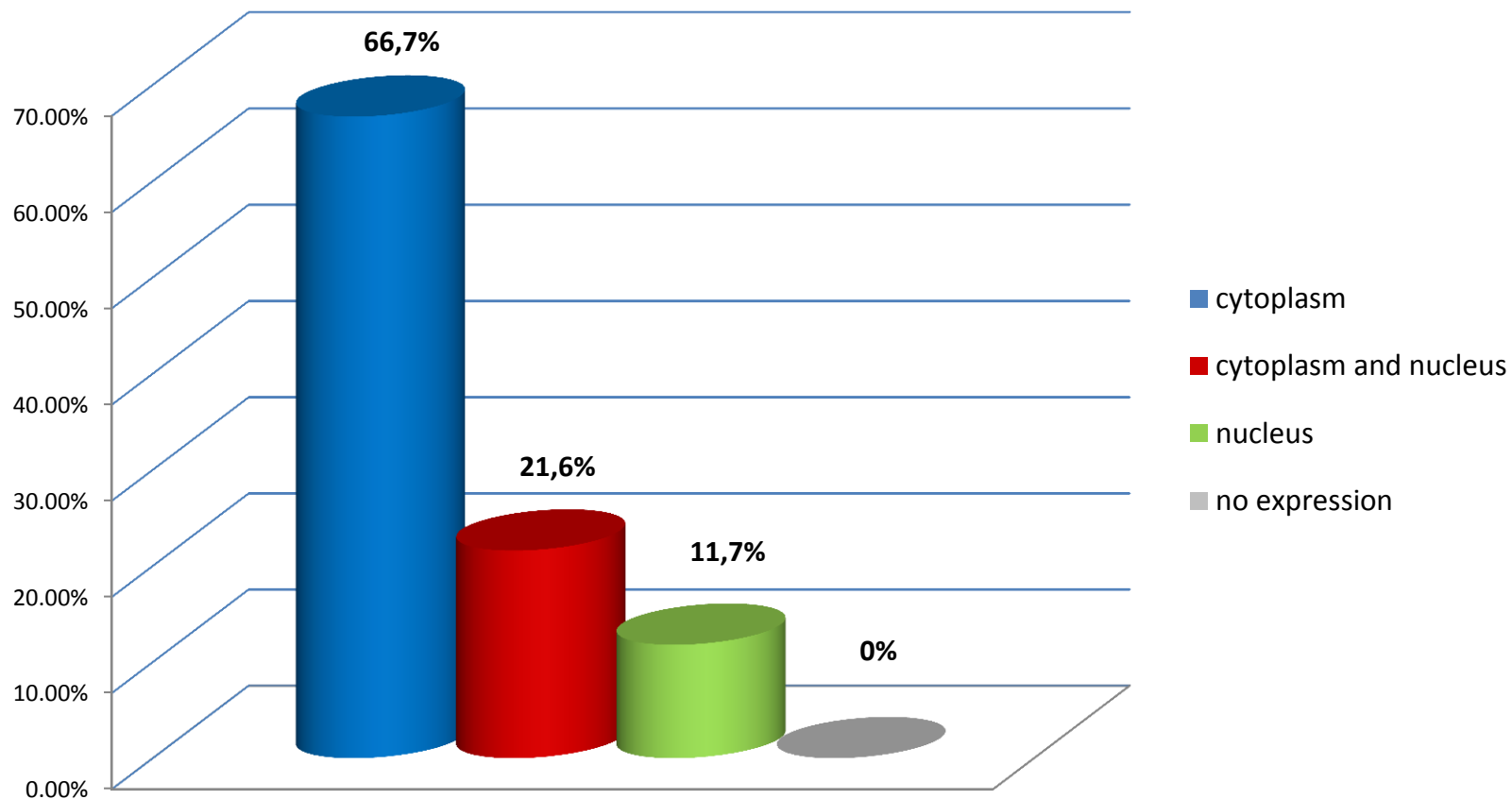**Comparation of relative LP values for axin1 and beta-catenin to cellular localization.**

Cellular localization	LP for beta-catenin*	LP for axin1*
Nucleus and cytoplasm	140.86	158.81
Cytoplasm	83.25	176.04
Nucleus	80.19	184.74

*The numbers represent LP =light permeability, so the protein levels are reversely proportional

The highest relative LP value of axin1 was measured when the protein was in the nucleus and it was accompanied with the lowest relative LP value for beta-catenin. The lowest relative LP value of axin1 was measured when the protein was localized in the cytoplasm and nucleus, and it was accompanied with the highest relative LP value for beta-catenin. This means that the quantity of axin1 is lowest in the nucleus, and is accompanied with the highest quantity of beta-catenin, while the quantity of axin1 is highest when located in the cytoplasm and nucleus, and is accompanied with the lowest quantity of beta-catenin.

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Pecina-Slaus N, Beros V, Nikuseva Martic T, Bulic-Jakus F. Genomic Instability of the APC Gene Found in Glioblastoma. In: Genome Research Advances. Ed. AP Bellini, Novascience Publishers, Inc. New York, 2007, pp.121-137

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Pecina-Slaus N, Nikuseva Martic T, Kokotovic T. AXIN1 (axin 1). Atlas Genet Cytogenet Oncol Haematol. 2011,15:933-937.
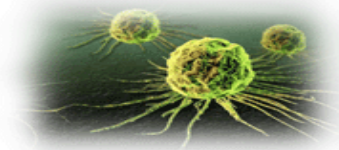URL: http://AtlasGeneticsOncology.org/Genes/AXIN1ID379ch16p13.html

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