The DNA-double strand break (DSB) repair has an essential importance for the genomic integrity maintenance. The main DSB repair pathways are homologous recombination (HR), non-homologous end joining (NHEJ) and single-strand annealing (SSA). The most important protein factors contributing to the maintenance of genomic integrity by direct participation in DSB repair are MRN, ATM, Rad51, BRCA1/2 and PALB2 in the case of HR; Ku70/80 DNA-PKcs, XRCC4 and DNA ligase IV in the case of NHEJ and Msh2-Msh3 and Rad1-Rad10 in the case of SSA. If mutated, these proteins can cause the inability to repair DNA lesions leading to a malignant transformation. The predominant phenotype manifestation of BRCA1/2 inactivation is the hereditary breast and/or ovarian cancer (HBOC). Mutations in ATM have been described as a cause of ataxia telangiectasia and inactivation of NBN gene (Nbs1 protein) causes the Nijmegen breakage syndrome. Other syndromes connected with defects in a DSB repair pathways are Fanconi anemia and Werner syndrome. Detail knowledge of DSB repair process is a mandatory for diagnostics and effective therapy of a number of malignances. An example of practical and clinically relevant utilization of current knowledge about the DSB repair process is the concept of a synthetic lethality as a specific therapy. This work summarizes our understanding about particular DSB repair pathways and indicates its practical usage in human medicine.